Reviewer’s report

Title: Displayed correlation between gene expression profiles and submicroscopic alterations in response to cetuximab, gefitinib and EGF in human colon cancer cell lines

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Reviewer: Helmout Modjtahedi

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Aberrant expression of the epidermal growth factor receptor (EGFR) with tyrosine kinase activity has been reported in a wide range of epithelial tumours and several EGFR inhibitors have now been approved for the treatment of human cancers. However, there is currently no clear marker for response to therapy with the EGFR inhibitors.

In this study, Solmi and colleagues evaluated the effects of EGF and two EGFR inhibitors namely anti-EGFR monoclonal antibody cetuximab and the small molecule EGFR TKI gefitinib on proliferation, cell cycle distribution and morphology of two human colorectal cell lines HT29 and Caco-2. In addition, gene expression profiles of these tumours were conducted following treatment with EGF and/or the EGFR inhibitors. They found that treatment of Caco-2 cells with EGF, gefitinib or cetuximab reduced the viability of these cells 24 hours post treatment. In contrast, HT-29 cells expressed higher levels of the EGFR but were not sensitive to treatment with EGF and/or the EGFR inhibitors. While EGF treatment induced internalisation of the EGFR in both Caco-2 and HT-29 cells the expression of EGFR was membranous in HT-29 and Caco-2 cells following treatment with the two EGFR inhibitors. In addition, treatment with the EGFR inhibitor in combination with EGF did not prevent the EGF-induced down regulation of the EGFR in both cells. Using scanning electron microscopy, HT-29 cells were found to be smaller than Caco-2 cells and their microvilli were shorter than those of Caco-2 cells. While EGF did not induce any morphological changes in HT-29 cells, it induced a lot of vesicles and very few microvilli in Caco-2 cells. Their results also indicated that HT-29 cells treated with cetuximab loss their contacts with each other and show a small number of microvilli. Caco-2 cells also displayed a very small number of microvilli which loss their erect position and form a star morphology post cetuximab treatment. Treatment of both HT-29 and Caco-2 cells with gefitinib resulted in some vesicles and a small number of microvilli. Finally, they found that up regulation by at least two fold of 49, 138 and 139 genes following treatment with cetuximab, gefitinib and EGF in both HT-29 and Caco-2 cells respectively. Based on global gene expression analysis, they found that gene expression profile (i.e. switched on/off genes) induced by cetuximab is similar for both HT-29 and Caco-2 cells.

Overall, this is an interesting study which could be strengthened following further clarification and response to the following points.
1) In general, the paper should be more focused and their major findings and implications highlighted in the results and conclusion part of the abstract. For example, the authors should give further details of their finding in the abstract section. In the results section of the abstract they have written that "In cell viability, Caco-2 showed statistically significant differences between controls and all treatments" with no further detail. This may be changed to something like "Treatment of Caco-2 with EGF or the two EGFR inhibitors produced a significant reduction in the viability of Caco-2 cells"

2) The EGFR expression was found to be higher in HT29 cells than in Caco-2 cells. Perhaps, the author could elaborate on why Caco-2 cells are more sensitive to treatment with both the EGFR inhibitors in the discussion section.

3) The expression profile of genes induced by cetuximab in both HT-29 and Caco-2 cells was found to be similar. However, only Caco-2 cells were growth inhibited by cetuximab. This in turn may suggest that gene profiling of tumours may not be useful in differentiating tumours which are sensitive from those that are resistant to treatment with the EGFR inhibitors. The authors may want to elaborate/respond to this in the discussion.

4) What were the reasons for conducting proliferation, cell cycle and morphological experiments at 24 hours post treatment with EGF and/or EGFR inhibitors? Due to tumour cell doubling times, it would have been better to investigate the effect of EGFR inhibitors on cell cycle distribution at longer times (e.g. 48 or 72 hours) post treatment.

5) It would be useful if the authors could give some indication of the number of cells in each flask prior to the addition of EGF or EGFR inhibitor (i.e. time zero). If such data are available, these should be added to Figure 1 as baseline number of cells at the start of the treatment.

6) Please check and label figures 2A, 2B, 3A etc correctly.

7) The SEM figures should be labelled completely by adding a-l to the figures.

What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.