Reviewer’s report

Title: Epidermal Growth Factor Receptor (EGFR) mutation analysis, gene expression profiling and EGFR protein expression in androgen-dependent prostate cancer

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Reviewer: Rosita Winkler

Reviewer’s report:

This is a descriptive work, showing EGFR protein expression and mutations in 100 human prostate cancers. Moreover, the genes expressed in EGFR positive and negative tumours were compared by microarray. The main finding of the paper is 8 novel mutations in EGFR tyrosine kinase domain. Indeed, EGFR expression in PCA has been reported previously, the expression data are very preliminary in my opinion.

Methods

Immunohistochemistry was used to detect EGFR protein, but the positive and negative controls are not mentioned.

The method used to amplify the mRNA for the microarray should be clarified – do they obtain 5 µg of mRNA from 5 µg of total RNA but how this was verified is not mentioned.

As mentioned below the discussion section is too long and some results are too preliminary to allow a definitive conclusion. The conclusion of the abstract should be more moderate.

The discussion section writing needs some editing.

Major compulsory revision

The manuscript contains weaknesses that should be corrected before publication.

1. Immunohistochemistry results are not convincing as presented. Figure 1 presents two magnifications of the same section. Several sections should be shown displaying different levels of positivity, and the cancerous and healthy cells should be pointed out. The positive and negative controls are not stated. Considering a tumour as “overexpressing” if only 1% of the cancerous cells are EGFR positive leads to over estimation of EGFR+ tumours. The literature reports different levels of EGFR expression in prostate cancers, but the lower threshold for overexpression is around 15% positive cells. What the authors mean by “positivity” or “overexpression” should be clarified. Indeed, healthy basal cells of the adult prostate express EGFR, so what is the cellular population to which the tumour is compared? How the number or proportion of positive cells is estimated? How many regions of the section have been examined? Does “overexpression” mean that the protein is detected or that the quantity is increased when
compared with the receptor expressed in healthy cells?

2. It is surprising that there was no difference between the genes expressed in EGFR positive and negative tumours. This could result from the over estimation of overexpressing tumours. Another possibility would be that the receptor is inactive. The authors should include an experiment showing the activation status of the receptor such as for instance phosphorylated EGFR or an activated target. The number of tumours is too small to allow for a valid statistically significant comparison between the genes expressed in mutated EGFR positive and negative tumours. To investigate the importance of the mutation the genes expressed differentially between EGFR positive tumours with or without the mutation should be compared. The functional significance of mutation in tumours not expressing the receptor is not clear.

3. Results in Figure 3 are difficult to understand. The legend does not indicate what the number on the Y axis mean nor to what P51, G65 etc are referring to. I think that the number of tumours is too small for a valid comparison. The authors should include additional samples to be able to obtain conclusive results. If this is not possible, I suggest to keep these results for another publication. Indeed, what conclusions can be drawn from the expression of FOXC1 – negative in EGFR- and in 2/3 EGFR+ cases? And from PSA negative in 3/5 EGFR - and in all 3 EGFR + cases?

4. qRT-PCR results are not significantly different among EGFR+ and EGFR- cases. This experiment must be repeated on a higher number of tumours.

Minor essential revision

1. The ground on which the tumours are considered as androgen dependent has to be indicated.

2. The description of the mechanism of action of drugs targeting EGFR in the 2nd paragraph of the introduction should be included in the discussion section. The discussion is too long. The language in this section should be corrected.

3. Has the DNA from healthy cells of the same patients been sequenced?

4. Since the extracellular domain of the EGFR was not sequenced paragraph 4 of the discussion section should be omitted or re-written. Eight of the 13 tumours bearing EGFR mutations were negative for EGFR expression. The significance of this is not discussed.

5. The novel mutations are the real interesting part of the manuscript. I would suggest to include in the discussion section a comment on the predictable consequences of the novel mutations on EGFR activity.

6. Discussion paragraph 6 – reporting the results of Cai et al on the functional consequence of the E804G mutation “most active and significant somatic missense mutation…” but in comparison with the other tested mutations. It should be clearly indicated.

7. Discussion end of paragraph 7: “An evaluation of EGFR…” should be deleted since this claim is not supported by enough data.

8. The end of the discussion section (paragraph 8 and following ) is too long. It
would be more correct to say that the expression data are too preliminary are the
significance of the results needs additional data.

Discretionary revisions
1. Could the region coding for the extracellular domain, which could identify the
EGFR\text{vIII} be sequenced?

Declaration of competing interests:

I declare that I have no competing interests.