Reviewer's report

Title: Upregulation of Claudin-4, CAIX and GLUT-1 in distant breast cancer metastases

Version: 3  Date: 18 August 2014

Reviewer: Céline Pinheiro

Reviewer's report:

In this manuscript by Jiwa LS. et al, the authors evaluated the expression of Claudin-4, GLUT1, CAIX, EGFR and IGF1R in a series of 97 primary breast cancers paired with metastases. Expression was assessed by immunohistochemistry and compared between primary tumors and the paired distant metastases. Some associations with clinicopathological features were also performed. This is a study with some relevance in its field; however, the authors should consider the following concerns:

Major Compulsory Revisions

1. When stating the aims of the work at the end of the Introduction, the authors state that they “evaluated how this would impact molecular imaging and targeted therapy”. However, to make this evaluation, the author would have to present results showing the use of these proteins for both ends. Please remove the sentence or rephrase, replacing “evaluated” by “hypothesised” or similar. Additionally, if the authors decide to maintain the sentence, the Discussion must be improved in accordance, as this impact in molecular imaging and targeted therapy is only slightly discussed.

2. Figure 1 must also include immunohistochemical results for EGFR and IGF1R.

3. Authors should provide tables showing the frequency differences that explain the significant p values obtain when evaluating the clinicopathological significance of the negative to positive changes.

4. Besides analysing the clinicopathological significance of negative to positive changes, authors should also provide associations between the clinicopathological features and the expression in primary tumors and metastasis, individually and discuss those results, in light of the results from the literature.

5. In Discussion, authors try to explain the association of CAIX with adjuvant therapy. Were samples collected before or after treatment? Did the authors take this important aspect in account when performing the statistical analysis?

6. The authors found an association of CAIX upregulation with younger patients and GLUT-1 upregulation with older patients. Besides the fact that the authors ignored the former in the Discussion, how do they explain that CAIX and GLUT-1, which are discussed simultaneously in result of their common features (hypoxia-induced proteins involved in the Warburg effect), show opposite
associations?

Minor Essential Revisions

1. In the Abstract, the frequencies of expression change (positive to negative and negative to positive) for each protein are not the same as shown in the Results section. Also, if (n.s) is used in negative to positive change, it should also be used in positive to negative change.

2. In the Introduction, the authors state that differences in protein expression when comparing primary tumors with their metastasis is “a process generally known as receptor conversion”. However, the only references provided are from the group and a quick search in the literature showed no other study referring to this phenomenon as “receptor conversion”. In fact, by reading only the Abstract, it is not clear what is “receptor conversion”, and the first relation made by someone who is not familiarized with this expression is with protein conformation, localization, degradation, but not level of expression. Please adjust the manuscript in accordance.

3. In the Material and Methods, the authors state that they selected 97 pairs from a group of 254. Which were the criteria for inclusion/exclusion? Also, why CAIX only present results for 52 cases?

4. Why metastatic carcinomas in the bone were marked with H&E if they were excluded from the study?

5. Please include the negative and positive controls used in the immunohistochemical procedure.

6. How was the semiquantitatively scoring performed? How was it used in the statistical analysis if cases showing plasma membrane in one out of three cores were considered as positive? Please include this information in the Material and Methods.

7. Figure 1 lacks scale bars and magnification.

8. Reference 28 does not shows that GLUT-1 indirectly regulates intracellular pH. Rather, it shows that GLUT-1 is expressed in the same tumors that express MCT1, an important pH regulator. Please adjust the Discussion in accordance with the reference.

9. In the Discussion, when referring to HIF-1#, please replace “which is maintained by IGF1R” by “which can be maintained by IGF1R”, as IGF1R is not the only HIF-1# inducer.

Discretionary Revisions

1. In the Discussion, the authors refer that the upregulation of Claudin-4 in breast cancer metastases occur especially in lymph node positive cases; however, this result is not provided in the Results. If a result is referred in the Discussion, it should be shown in the Results.

2. In the Discussion, the authors refer the preanalytical an analytical variability that should be taken into account. However, they do not further discuss this matter. Can the authors elaborate more on this aspect?
Minor issues not for publication

1. In the end of the first paragraph of the Abstract, there is an extra space after the text.

2. In the Abstract, “upregulation” and “negative to positive conversion” are used to transmit the same idea. Please refer equally to avoid reader’s confusion.

3. In the last paragraph of the Introduction, a parenthesis is opened but not closed.

4. In the Results, second paragraph, Claudin-4 results for positive to negative changes are inverted when compared to the other proteins (percentage in parentheses and absolute numbers outside parenthesis).

5. The statistical analysis performed was not of correlation but of association. Please replace “correlation” by “association”.

6. Please adjust the last sentence of the Results as it is not clear.

7. A reference is lacking from the first paragraph of the Discussion (Such conversion was previously described for ER, PR and HER2 receptors).

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

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.