Reviewer's report

Title: Production and Characterisation of monoclonal Antibodies against RAI3 and its Expression in human Breast Cancer

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Reviewer: Nicolas de Roux

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RAI3 is an orphan g-protein coupled receptor expressed in several tissues which has been involved in cell proliferation by unknown mechanisms. Development of RAI3 antibody might be a powerful tool in clinic as well as in therapeutics. In this paper, Jörißen et al report characterisation of monoclonal antibodies against RIA3 and quantitative analysis of RAI3 expression in human breast cancer.

Methods are described in great details, which is necessary for the understanding of the procedures. Results are also well presented and described but the conclusion which affirm that Mab 24 2.3 is a highly specific antibody against RAI3 is premature. Several new controls are requested. This point is critical because the authors propose to use this antibody for additional clinical research in cancer.

Major Compulsory Revisions

Supplementary information or controls which are required to definitively prove RAI3 specificity.

- Supplementary informations
  o In RAI3 production, the authors used a RAI3 cDNA cloned in frame with GST and His tag, produced inclusion bodies, cleaved the GST tag with thrombin, and purified the recombinant RAI3 using Ni-NTA superfllow in presence of Glutathione. The authors must explain why they used a plasmid expressing RAI3 in frame with GST? Why they did not use a recombinant RAI3 in frame with His Tag only.

- Supplementary controls.
  o The authors used competitive-ELISA to affirm specificity of their antibody and then expressed RAI3 in HEK293 to perform western blot. Western blot did not reveal any band in untransfected HEK293 cells. An additional control by immunohistochemistry must be performed on transfected and untransfected HEK293 cells. It will be interesting to perform this control in permeabilized and unpermeabilized cells.
  o Why the authors did not found any band in HEK293 untransfected cells whereas in introduction they reported a paper indicating that siRNA against RAI3 reduces HEK293 cell growth (ref 5). This discrepancy with literature results must be explained or discussed.
o In the western blot, they explained their multiple bands by RAI3 proteolysis. It could be an explanation, however, cross hybridization with other molecules is another explanation. It is quite easy to test this hypothesis by performing siRNA experiment in breast cancer cell line expressing RAI3. These controls are absolutely necessary before performing any quantitative analysis of RAI3 expression using tissue microArray.

o The authors has performed cDNA dot blot to analyse RAI3 expression. It is an old technique for such purpose. Why they did not use quantitative RT-PCR which is clearly more accurate? They don’t show any northern blot on total RNA extracted from breast cancer? How they analysed specificity of cDNA probe? Did they use a full length cDNA or only a portion? They must precise negative controls used in this experiment.

o Immunohistochemistry analysis on breast cancer is not convincing. Why they use a non-specific negative control by omitting the first antibody. When immunogen is available, the true negative control is the immunoneutralisation of the primary antibody. This analysis must be performed.

o Without these additional controls, results expressed in table 2 are not relevant and must be removed from the manuscript.

Minor Essential Revisions
Page 19 : “transcription of mRNA” should be read “traduction”.

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

**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.