Reviewer's report

Title: Ephrin-A1 Inhibits NSCLC Tumor Growth via Induction of Cdx-2 a Tumor Suppressor Gene

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Reviewer: Christine Sers

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The manuscript of Bhagyalaxmi A. et al describes EphA2 receptor (EphA2) activation through the ligand EphrinA1 (EFNA1), a subsequent induction of cdx-2, a tumor suppressor gene, which finally leads to decreased cell proliferation and tumor growth via suppression of the tight junction protein Claudin-2.

In general, the manuscript is weak with regard to the content. The authors describe a potential role for EphA2 signaling in lung malignancies, yet the complete manuscript deals with just one single cell line and nothing has been tested in other NSCLC cells. EphA2 signaling is described to play a major role, however this is not shown, because no phosphorylation of the receptor or signaling molecules has been tested as an indicator of pathway activation. What is the functional relationship between cdx and Claudin-2? EphA2 signaling is likely to affect a whole lot of genes, why should cdx be the one-and-only Claudin-2 master regulator?

In general the manuscript lacks several controls, e.g. there is no Western blot or ELISA for EFNA1 overexpression is shown. In addition, there is no control for all of the siRNA effects. Several figures are documented quite poorly and don’t show scientific standards. For example, Figure 1D shows a cut-out lane for EphA2. The blot is very hard to judge. I don’t see an overexpression of EphA2. On the protein level the Western Blot for Claudin (which is nice) does not provide any difference between the control and the EphA2 transfected cells; obviously this only works at the mRNA level, however the authors don’t comment on this. Paragraph 1 and 2 should be combined; they describe the Figure 1 in a lengthy manner. In Figure 3C the Y-axis does not contain a legend. What do we see there? If it is optical density, what is it normalized against? Most of the (control) blots are heavily overloaded and individual lanes cannot be judged reasonably. In Figure 2B the downregulation cannot be seen due to the scale of the X-axis. Could it be that Figure 1 and Figure 2 are very similar? Just the one is a Western blot and the other is immunofluorescence. What kind of additional information do we get from Figure 2? In Figure 5C the authors shown no significant increase in proliferation upon siRNA of cdx2 following treatment with EFNA1 ligand or transfection with EFNA1 vector. In the result chapter, the author describe that there is a significant increase. This discordance should be clarified. Further, proliferation of cells with cdx overexpression and cxd-2 siRNA should be shown to analyze, whether proliferation of cells depends directly on the cdx tumor
suppressor gene activated via EFNA1. In Figure 6 letters are missing which was indicated in the text.

The methods are not described in detail, but the authors describe the same for EphA2 and EFNA1 vector design and transfection in two paragraphs. This should be combined.

In general, there are a numerous spelling and grammar mistakes and also the format of the manuscript is not acceptable. For example, it is spelled claudin not caludin or in Figure 2 it is EFNA1 not EFA1. It would be helpful to use a word processing program. In the discussion there is even a reference lacking (insert reference), maybe the authors submitted a preliminary version?

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Not suitable for publication unless extensively edited

**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