



## **SDI Review Form 1.6**

### **PART 1:**

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| Journal Name:            | <a href="#">International Journal of Biochemistry Research &amp; Review</a>                                                    |
| Manuscript Number:       | 2013_IJBcRR_3847                                                                                                               |
| Title of the Manuscript: | The Soluble Ectodomain but not Intracellular Domain of Jagged1 Regulates the Expression and Proteolytic Shedding of E-Cadherin |

**General guideline for Peer Review process is available in this link:**

**(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)**

- This form has total 7 parts. Kindly note that you should use all the parts of this review form.



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### **PART 2: Review Comments**

|                                            | <b>Reviewer's comment</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | <b>Author's comment</b> <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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| <b><u>Compulsory</u></b> REVISION comments | <p>Since a recent publication has identified the cleavage site in human jagged-1 (distinct from constructs used in this report) and shown that soluble jagged-1 is sufficient to induce Notch signalling, this report provides no significant new insights into jagged-1 biology. The relevance and significance of their observations are also unclear.</p> <p><b>1.</b> The suggestion in this manuscript that soluble jagged-1 is capable of inducing signalling is not novel. A recent paper has much more clearly demonstrated that soluble jagged-1 is sufficient to induce Notch signaling (Lu, Cancer Cell, 23:171-185, 2013).</p> <p><b>2.</b> The effects of overexpression of different jagged-1 constructs on E-cadherin mRNA is not impressive and has not been quantified using a reliable approach, only with a semi-quantitative method. The data shown are not sufficient to draw conclusions regarding 'bi-directional' regulation of E-cadherin by JICD.</p> | <p>Not correct - no previous report addresses the independent effects of sJag1 and JICD directly on E-cadherin expression. Granted there are reports linking soluble Jagged1 to Notch signaling (some of which were already stated in the manuscript) but the molecular determinants of Jagged1-mediated changes in E-cadherin are of particular importance in relation to EMT.</p> <p>No, but this is not really the crux of the paper! As stated above, this paper has considerable novel implication for EMT in relation to the mechanisms of Jagged1-mediated E-cadherin expression.</p> <p>What does 'impressive' constitute exactly from a scientific point of view? Such changes are obvious and statistically significant. However, in support of the reviewer, a second reviewer did request, quite rightly, RT-qPCR data in place of the semi-quantitative data. In actual fact, this data has thrown up an interesting result in relation to the effect of the JICD construct on E-cadherin RNA levels. Clearly there was a 'plateau' effect in existence in our previous semi-quantitative RT-PCR data. Although, this change does not translate through to E-cadherin protein levels there is a general 'trend' for increased soluble E-cadherin in the conditioned medium of JICD-transfected cells which was</p> |



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|  | <p><b>3.</b> The relevance of the longer soluble form of jagged-1 evaluated in this study is questionable since two groups have independently demonstrated that soluble jagged-1 is cleaved c-terminal of E1054 (Lu, Cancer Cell, 23:171-185, 2013; Coglievina, BBRC, 432:666-671, 2013), not further C-terminal.</p> <p><b>4.</b> It is not clear whether replicates used for quantification in Figure 3 and other figures are valid biological replicates. As recently reviewed (Cumming G et al., J Cell Biol, 177:7-11, 2007), replicate experiments, not replicate wells, are required for evaluation of statistical significance.</p> | <p>commented on by another reviewer. We have added discussion of this and the RT-qPCR data to lines 335-343 of the Discussion.</p> <p>We thank the reviewer for raising this point as these two references will be of great use to our future Jagged1 experiments. In the context of the current manuscript, we have added text (lines 172-188) by way of a comparison of our soluble construct to the published cleavage site. However, as we have stated in the text, the chances of the extra 13 amino acids at the C-terminal end in a non-functionally important part of the Jagged1 molecule impacting on our results are vanishingly small. As such, we would hardly consider the relevance of our construct 'questionable'.</p> <p>Yes they are - in fact they have even been performed by different members of my research team. They are as independent replicates as can possibly be.</p> |
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| <p><b><u>Minor</u></b> REVISION comments</p>   | <p>1. Why were the westerns of lysates not normalized to actin levels? Also Westerns need to be developed so that the control actin can be accurately quantified to use for normalization.</p> | <p>Because we conducted actin blots for each experimental replicate and there were negligible differences between loadings in any of the experiments. Besides the standardization of results to actin rather than total protein levels is a somewhat arguable practice - there is always the small possibility that apparent changes in a 'house-keeping protein' specific expression level might be brought about by the transfection of a particular protein itself.</p> |
| <p><b><u>Optional/General</u></b> comments</p> |                                                                                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |