



SDI Review Form 1.6

PART 1:

Journal Name:	International Journal of Biochemistry Research & Review
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.



SDI Review Form 1.6

PART 2: Review Comments

	Reviewer's comment	Author's comment (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)
Compulsory REVISION comments	Major points. It seems to me that some of the conclusions (the title in the first instance) are not fully supported by the experimental results shown, and I would like the authors to comment on the following points. Line 286: <i>“levels of the protein remained unaltered in lysates from JICD transfected cells”</i> Line 289: <i>“The levels of soluble E-cadherin in the conditioned medium from JICD-transfected cells were unaltered”</i>. The same statements are repeated in the Discussion section (lines 340-342) and in the Conclusion (lines 350-352). From Figure 3A, I would say that the level of E-	The title has been altered to better reflect the results obtained in the study I think the key point to pick out from this section of comments is that there is a <u>trend</u> for the JICD to enhance levels of soluble E-cadherin in conditioned medium (I don't think this can really be argued in cell lysates). The problem is that this is all that it appears to be i.e. a 'trend'. As the reviewer will see we already have 6 independent repeats of this particular experiment and cannot attach any significance to this change (I could add in another two independent repeats from a recent project student's work but this still does not make the differences significant).



SDI Review Form 1.6

	cadherin, as detected by immunoblotting, is in the lysate slightly higher in cells over-expressing JICD than in the control (although the standard deviation is high) and, in the medium, that it is nearly two times higher in cells over-expressing JICD than in the control (Figure 3C). Overall, it seems that over-expression of JICD is associated with an increased level of E-cadherin, over-expression of sJAG1 is associated with a decreased level of E-cadherin, with over-expression of full length WT-JAG1 somewhat in the middle (but closer to sJAG1). This trend seems to be confirmed by mRNA levels for E-cadherin, with JIICD > WT-JAG1 > sJAG1. Is it possible that over-expression of the intra- and extra-cellular parts of Jagged1 have opposite effects? In the Discussion, the authors comment on the correlation between Jagged-1 over-expression, E-cadherin levels, and Notch signaling. Actually, no	However, interestingly, the quantitative RT-PCR data that we have inserted into Fig.4 (in place of the semi-quantitative data; as requested by another reviewer), does show a highly significant increase in E-cadherin mRNA in the JICD-transfected cells. Unfortunately, this does not seem to translate through to the protein level. It is possible that E-cadherin protein synthesis levels <u>are</u> increased in the cell but so too are levels of its shedding (if one is to believe the 'trend' in the protein data) thereby negating any E-cad changes in cell lysates. So, in response to the reviewer's comments, all we can do is comment on this possibility in the Discussion (which we have done; lines 335-343). We agree that we have not presented direct evidence of alterations in Notch signalling in the current manuscript. However, given that others have previously linked
--	--	--



SDI Review Form 1.6

	experimental evidence of Notch signaling activation is reported, nor Notch activation is measured in any way. Therefore, I would limit the discussion to what is actually measured, i.e. levels of E-cadherin. As Notch receptors as well as Notch ligands and E-cadherin all undergo ADAM-mediated shedding, is it possible that what is observed is actually a competition of different substrates for the same ADAM enzyme?	alterations in <u>full-length</u> Jagged1 to Notch-mediated changes in E-cadherin expression, we still consider it pertinent to include the discussion of such possibilities in the current manuscript. However, we have 'toned down' to a degree our arguments in relation to Notch regulation at various points within the manuscript. A possibility if it weren't for the fact that sJAG1 exhibits a very similar effect to its full-length counterpart. Although this construct might well retain the ADAM cleavage site it is unlikely to be an effective substrate as it would seem that these enzymes require their substrates to be inserted into a lipid bilayer for effective cleavage to occur. The fact that a recombinant soluble construct mapping the juxtamembrane region of Jagged1 is cleaved by recombinant ADAM17 catalytic domain in Coglievina et al (Biochem. Biophys. Res. Commun. 2013, 432: 666-671) bears no relevance in this respect as such soluble peptides are commonly cleaved where the corresponding 'full-length' soluble ectodomain is not.
--	--	--



SDI Review Form 1.6

Minor REVISION comments	The precision given for expression levels (%) (two decimals) is highly optimistic. I think it would be more sensible to round the values to the closest integer number. Line 169: “... <i>the exact ADAM cleavage site within Jagged1 has not been determined</i>”. Actually, it has been recently reported (Biochem. Biophys. Res. Commun. 2013, 432: 666-671) that Jagged-1 (in fact, a recombinant protein corresponding to the juxtamembrane region) is cleaved at E1054 by ADAM17 catalytic domain. This should be mentioned in the text, or as a footnote added in proof.	These values have now been rounded to the closest integer number. Thank you for pointing this out - it is most useful to other areas of our Jagged1 research and we have incorporated suitable comments on this in the relevant parts of the current manuscript. There is also another article (Lu et al. 2013) that has been mentioned to us by another reviewer in a similar respect. We consider it vanishingly unlikely that the incorporation of an additional 13 amino acids at the C-terminus of our construct in a non-functional region would impact on our results. However, the reviewer is absolutely right to point this out and we have incorporated text to expand upon this point in lines 172-188 of the current version of the manuscript.
---------------------------------------	---	--



SDI Review Form 1.6

	In Figure 2, I would swap the position of panels D and E, so that in the upper part we would have the results of the anti-C-terminal Jagged antibody, in the lower part those for the anti-N-terminal Jagged, with the left part for the lysate, and the right part for the medium. In panel E, what is the “+’ve Control”? In Reference 12, the publication year is missing.	Noted but this would make the flow of the results commentary more illogical. It makes sense to deal with both blots for the lysates first (i.e. panels A and B) and then both blots for the medium (i.e. panels D and E). This is stated in the main text body but we've added the information to the Figure legend in order to clarify the matter. Adjusted accordingly.
<u>Optional/General</u> comments		