



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:

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- 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	There is no acceptable reason to use "semi-quantitative" RT-PCR analysis of E-cadherin expression levels. These experiments should be repeated using quantitative RT-PCR. Statistical significance testing of multiple groups should be by ANOVA, not by iterative Student's T tests.	The reviewer is absolutely correct to say this and our new results using RT-qPCR clearly vindicate the reviewer's comment in that they have shown up an interesting difference in relation to the effect of the JICD construct on E-cadherin RNA levels. Clearly a 'plateau' effect was evident in our original semi-quantitative RT-PCR data. In fact we feel that these data have added significantly to the interest of the manuscript. So this data has been inserted into Fig.4 in place of the original data. Unfortunately, the increase in E-cadherin RNA in response to JICD transfection does not seem to translate to the protein level. However, a general 'trend' in increased E-cadherin shedding in these cells was commented on by another reviewer so this new RT-qPCR data has allowed us to expand the discussion in relation to this latter comment (lines 335-343). Note that we have also added two additional authors to the manuscript - these were the colleagues who, at short notice, conducted the RT-qPCR experiments. The methods section has also been updated accordingly. In terms of statistical testing the important issue here is whether any of the Jagged1 construct-transfected cells differ in terms of E-cadherin from the mock-transfected cells. As such, each Jagged1 construct-transfected cell line is



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	on Line 197: should read "(Fig. 2A)"	analysed in a paired test against the mock. Error on what was line 197 is now corrected.
<u>Minor</u> REVISION comments	The authors should state whether there was any change in the morphology of the cells expressing the three different JAG1 constructs. on Line 287: transfected cells (add space) I really doubt that the levels of E-cadherin protein reduction are significant to 4 digits (for example, 37.66% on line 286)	This is a fair point given the role of E-cadherin expression in EMT. We did not see any changes in HEK cell morphology. However, given the nature of this cell line (a heterogeneous population of cells being derived from neural crest cells, neurons and glia and many other subpopulations of cells) there is no evidence to suggest that, in terms of morphology, cancer epithelial cells would behave like HEK cells in relation to E-cadherin expression levels. This is why we have refrained from commenting/extrapolating in relation to this observation. Error on what was line 287 is now corrected These values have now been rounded to the closest integer number in accordance with a similar statement/request made by one of the other manuscript reviewers.
<u>Optional/General</u> comments	Straightforward, generally well-performed study of the effects of expression of several JAG1 isoforms on E-cadherin expression and processing.	