



SDI Review Form 1.6

Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2014_IJBcRR_14873
Title of the Manuscript:	The E1 Copper Binding Domain of Full-length Amyloid Precursor Protein Promotes Epithelial to Mesenchymal Transition in DU145 Cells in an Isoform-specific Manner
Type of the Article	

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: 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	1. The key funding of APP₆₉₅ on EMT are based on the light microscopy and dendricity analysis. I am not sure if this method is enough powerful and convictive for the EMT assay. Whatever, I think you should describe in detail or verify this by citing paper. In addition to this, the author needs another classic or traditional method to verify this important result, such as migration or invasion tranwell experiments. 2. The results show that both the extracellular E1 copper binding domain and the cytosolic domain tyrosine687 account for the APP₆₉₅ function on EMT. However, the title of paper did not accurately describe the key finding. Why did the author only emphasize the E1 copper binding domain in title? 3. There are some confusing about the function of APP isoform. According to the results, the E1 copper binding domain and tyrosine687 of APP₆₉₅ is key domain to promote EMT. However, the APP₇₅₁ or APP₇₇₀ also consist the two domains. Why they did not have the same function as APP₆₉₅. The author should gave the reasonable explain about this phenomenon. This should be addressed in the discussion.	1. The fact that full-length APP affects EMT is verified by dendricity analysis, E-cadherin and vimentin. The crux of the paper is based on the molecular prerequisites within the APP molecule required for these changes. It has already been shown in a previous publication that the changes in E-cadherin/vimentin brought about by APP correlate with changes in invasion/migration. We would simply be replicating already published data by doing this - we do make reference to this paper in the manuscript - e.g. line 62 - Miyazaki et al. [11] 2. The title becomes a real mouthful if you include all this information. We have stated in the title that it is 'Full-length' APP that is required for this effect which implies that the cytosolic domain is a prerequisite. Other information is made clear in the abstract. 3. Clearly, whilst the E1 CuBD and cytosolic tyrosine are prerequisites for EMT there must be other factors specific to APP₆₉₅ involved in the process. To comment on these would be purely speculative. However, we have highlighted this point in the conclusion by adding the text: ' However, it is clear that, whilst these factors are prerequisites for APP-induced EMT, there must be additional properties specific to APP₆₉₅ that participate in the phenomenon given the fact that the larger APP₇₅₁ and APP₇₇₀ isoforms do not act in a similar manner.'
Minor REVISION comments	1. Fig 1, although the author addressed that it was not necessary to perform real time quantitative PCR, I thinks this results is important for demonstrating the expression	1. We specifically went to considerable lengths in the text to comment on the fact that these data were not supposed to be particularly quantitative rather



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	of APP isoforms in prostate cancer cell line, so it is recommended to apply the real time quantitative PCR to quantify the expression of APP isoform. 2. Table 1, should analyze the statistical significant data between a series APP molecular constructs and APP695, which will show the role of key domains. 3. N-cadherin is the key marker of EMT, so in Fig 2B the author should add the N-cadherin protein expression data by WB.	qualitative. The experiments were purely performed in order to get a handle on the different types of APP isoforms present in DU145 cells. There is no need for real-time PCR at all. 2. This is just another way of comparing the various constructs to the mock-transfected cells. If wild-type APP shows a difference and a construct of APP lacking the cytosolic domain, for example, doesn't then it is clear that the cytosolic domain is involved in the phenomenon. The way the data is analysed is entirely appropriate. 3. Whilst we recognise that the E-/N-cadherin axis is important in EMT we feel strongly that the combined strength of our morphological data and the E-cadherin and vimentin data are sufficient to prove a change in EMT.
<u>Optional/General</u> comments	The authors in this manuscript describe a new function for the amyloid precursor protein (APP), and identified the key functional domain of APP on EMT of prostate cell line by isoform-specific manner. Using a range of APP molecular constructs, they demonstrate that the full-length 695 amino acid isoform (APP₆₉₅) can promote EMT via a mechanism requiring an intact extracellular E1 copper binding domain and tyrosine687 within the cytosolic domain of the protein. The data are new and original. These findings deepen the understanding of how APP promote EMT, and therefore have impact on the how targeting APP contributes to therapeutic strategies for prevention of prostate cancer metastasis. However, there are a couple minor points that should be addressed. Otherwise, this paper is suited for this journal.	Thank you for your comments.