Reviewer’s report

Title: Amyloid-beta Precursor Protein Promotes Cell Proliferation and Motility of Advanced Breast Cancer

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Reviewer: Kuniko Horie-Inoue

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The authors investigated the effects of APP knockdown on breast cancer cells. Their findings are consistent with previous findings by others, reporting that APP could contribute to the tumorigenesis of several solid tumors including breast cancer. They claimed that APP knockdown would induce p27 and caspase-3-mediated apoptosis. They also claimed that APP knockdown would reduce AKT activation in response to IGF-1. The manuscript is well described based on solid experiments, although some of the points need to be improved to define the role of APP in breast cancer biology.

Major points

1. They performed loss-of-function study of APP but not gain-of-function study in breast cancer cells. To confirm their findings in regard to APP knockdown, it is required to investigate the effect of APP overexpression on the biology of breast cancer cells.

2. Because they showed that APP was differentially expressed in M-II, -III, -IV cells compared to M-I cells, most of the functional studies for APP should be performed in at least 2 cell lines with different APP expression (e.g., M-IV versus M-I), rather than in MDA-MB-231 cells alone.

3. Most of the functional experiments were performed by shAPP-7 alone in MDA-MB-231 cells. The similar experiments for Figures 1, 2, & 5 need to be conducted by shAPP-5 or by more than 2 siRNAs against APP in at least 2 cell lines.

Minor points

1. shAPP-5 seems to be less efficient than shAPP-7 in regard to the effect of APP knockdown on APP protein expression (Figure 2A). Why the percentage of apoptotic cells in shAPP-5 was higher in cells treated with shAPP-5 than in those with shAPP-7? If their efficiency can be easily altered with different multiplicity of infection, they should monitor the concentrations of shAPP lentiviral particles such as through the integration of a reported gene (e.g., GFP) in the viral vector into the transfected cells.

2. For cell growth assay, MTT assay should be performed in a time-dependent manner, such as day 1 to day 7 after the transfection.
3. shAPP-7 significantly reduced the growth of MDA-MB-231 cells in conventional culture system (Figure 1E) and in xenograft study (Figure 4D), although the inhibitory effect of shAPP-7 on the cell growth was not so remarkable in 3D Matrigel on-top assay in Figure 4B. Please explain the reasons for the data.

4. A recent report by Goodarzi et al. (Nature 2014, doi: 10.1038/nature13466) showed that APP is rather a metastasis suppressor gene in breast cancer together with ZNF395. The findings by Goodarzi et al. seem to be controversial to the results by this group, as they described that “advanced breast cancers with knockdown of APP are more prone to enter into apoptosis” in the present study (lines 16-17, page 18). Please explain the difference of APP function between the 2 systems.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

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