Author's response to reviews

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

Authors:

Seunghwan Lim (sxl269@case.edu)
Byoung Kwon Yoo (bk1003@gmail.com)
Hae-Suk Kim (hxx119@case.edu)
Hannah L. Gilmore (hannah.gilmore@uhhospitals.org)
Yonghun Lee (yxl593@case.edu)
Hyun-pil Lee (hxl204@case.edu)
Seong-Jin Kim (kimsj@cha.ac.kr)
John Letterio (john.letterio@case.edu)
Hyoung-gon Lee (hyoung-gon.lee@case.edu)

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Author's response to reviews: see over
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Dafne Solera, Ph.D.
Executive Editor
BMC-Cancer

Dear Dr. Solera,

Thank you for the comments regarding our manuscript #1806927553127073, entitled “Amyloid-beta precursor protein promotes cell proliferation and motility of advanced breast cancer”. We have carefully read and addressed the reviewers’ comments and the critiques and responses are outlined below point-by-point.

**Reviewer: Kuniko Horie-Inoue**

**Major points**

1. It will be better to perform the gain-of-function study of APP in breast cancer cells with a milder phenotype (e.g., MCF7 or M-I cells), rather than those with an aggressive phenotype. Alternatively, the addition of exogenous APP to breast cancer cells would provide useful information as Goodarzi et al. performed in their study. The authors mentioned that APP has no oncogenic enzyme activity that can force normal breast epithelial cells to become cancerous cells in their responses, although previous literature showed that exogenous APP promoted the proliferation and migration of fibroblasts [Saitoh T et al., Cell, 1989] as well as cancer cells [Pietrzik Cu, et al., PNAS, 1998; Hansel DE et al., 2003]. Thus, it will be worth investigating whether the overexpression or addition of APP contributes to the progression of breast cancer biology.

- As suggested by the reviewer, we performed the experiment to test if exogenous APP enhances motility in APP knockdown MDA-MB-231 cells. Specifically, either parental (control) or APP knockdown (shAPP-7) MDA-MB-231 cells were incubated in serum-free medium (SFM) or SFM with soluble form of APP (sAPP, 0.1 µg/ml, R&D system) for 24 hours. The wound healing assay was performed (n=3) and the distance between wound edge of each well was measured with the IncuCyte Zoom microscope and its image analysis software. As demonstrated in our manuscript, the reduction of APP in MDA-MB-231 cells significantly reduced cellular motility.
compared to wild-type control cells (p<0.05). However, this effect was not rescued by the sAPP treatment in either control or APP knockdown cells, suggesting reduction of sAPP is not directly or solely responsible for the reduced motility in MDA-MB-231 cells. This result further supports the notion that the function of APP in cancer progression is multifactorial and cellular context is a major determinant of its regulatory function in cancer progression. Therefore, as discussed in the manuscript, further study is required to address this phenomenon and identify the further mechanism how APP regulates cancer development which will be our future research goals.

I would like to thank you for the opportunity to clarify the manuscript according to the reviewers’ comments.

Sincerely,

Hyoung-gon Lee