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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Biological Chemistry
ESPS manuscript NO: 20164
Title: High levels of homocysteine downregulate apolipoprotein E expression via NF-kB
Reviewer's code: 02446638
Reviewer's country: United States
Science editor: Yue-Li Tian
Date sent for review: 2015-06-02 08:16
Date reviewed: 2015-06-11 03:15

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[Y] Minor revision
[ ] Grade E: Poor		[Y] No	[ ] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

This manuscript is focused on determining the effect of high homocysteine levels on apoE expression, and the signaling pathways involved in this gene regulation. The authors have found that high levels of homocysteine are associated with a decrease in apolipoprotein E expression. These response were accompanied with modulations in NF-kB and MEK. The authors suggested that the homocysteine-mediated decrease of apoE expression in peripheral tissues may aggravate atherosclerosis, neurodegenerative diseases and renal dysfunctions. This is an interesting study where the experiments are reasonably well planned and executed. However, the following concerns need to be addressed. 1. The manuscript needs to be careful edited for English language and presentation. 2. The figure legends need to be expanded to provide some details. 3. The figures need to include information about statistical significance.

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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Biological Chemistry
ESPS manuscript NO: 20164
Title: High levels of homocysteine downregulate apolipoprotein E expression via NF-kB
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Science editor: Yue-Li Tian
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CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[ ] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[ ] Minor revision
[ ] Grade E: Poor		[Y] No	[Y] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

Hyperhomocysteinemia (HHcy) is known a risk factor of atherosclerosis while ApoE is a protective player. Trusca et al in this manuscript investigated how HHcy downregulates ApoE, thus potentially contributing to the development of atherosclerosis. They collected data showing homocysteine (Hcy) regulation of the apoE promoter and expression using cell culture model. They observed that high Hcy cose downregulated Apo E mRNA and protein in cultured RAW 264.7 and HEK293 cells and further found that activity of the ApoE proximal promoter (-500/+73)-luciferase reporter is regulated by Hcy in transient transfected cell lines. Their data potentially provide insight of Hcy related atherosclerosis and thus contribute to our understanding of atherosclerosis development. However, this manuscript suffers from several unclear statement and insufficient experimental design, which should be fixed before considered for publication. Specific comments: 1. Hcy is a potential excitatory neurotransmitter and binds to the NMDA subtype of glutamate receptor. NMDA receptors are expressed in the neural cells as well as in some non-neural cells. However, HEK293 cells do not express these receptors and thus have been widely used to evaluate the function of NMDA receptors after force expression by transfected construct, vioron or mRNA. Based on these facts, authors should

explain how Hcy acts on HEK cells and then the MAPK pathways, NFkB factor and eventually downregulated the ApoE promoter. 2. Introduction should be provided with information relevant to the studies rather than systematically reviewing the background. Some irrelevant paragraphs should be removed, e.g., the last three sentences in the 1st paragraph on page 4; the 2nd and 3rd paragraphs. 3. Some concepts or English writing should be clarified. Examples are listed below. 1) In the result section of abstract, transfection method does not need to be explained, but the evidence how MAPK activates NFkB et al should be described. 2) In the 3rd sentence of methods of abstract, "plasmids encoding … to drive" does not make sense because first, DNA sequence can encode mRNA for polypeptide and thus be included or constructed in a plasmid; second, promoter sequences instead of a plasmid can be used to drive a reporter gene. 3) In Material and Methods (p. 7), Information of EuroClone is unclear with MI which should be fully spelled out. 4) Source information of some critical materials should be provided, such as, Hcy, expression constructs of DD Ikb, IKKb, and synthetic (likely fused) p65-p50. 5) Information of experimental repeats should be provided. 6) Figures should be prepared consistently, e.g., Fig 1 should have error bars and Fig 2 should be presented in the same format, i.e., bar graph. 7) Statistic method should be provided 4. What is Hcy concentration in Hyperhomocysteinemia? Is it comparable to the concentrations used in cultured cells here? Fig 1B is one western blot. Is this only result? If so, this experiment should be repeated at least six times. In addition, longer exposure of apoE blot to X-ray film should be shown to exclude the possibility that apoE promoter is completely silenced. Hcy concentrations used for RT-PCR and protein assay should be the same. 5. Luciferase activity in transient transfected cells is normalized to β-galactosidase as described in the methods. However, information of the β-galactosidase in transfection is missed. Importantly, the promoter that drives the β-galactosidase expression may be regulated by Hcy and this possibility should be excluded. This is particularly critical when Hcy treatment is overnight. This concept should be applied to Fig 2 and Fig 3. This means that whether Mapk pathway acts on the promoter driving β-galactosidase expression and thus change relative luciferase activity should be addressed. The best model is the stable transfectants in which total cellular DNA can be used to normalize luciferase activity and no second report