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ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Biological Chemistry
ESPS manuscript NO: 20957
Title: Activation of Bmp2-Smad1 signal in Raf-induced senescence
Reviewer's code: 02608938
Reviewer's country: United States
Science editor: Xue-Mei Gong
Date sent for review: 2015-07-16 17:32
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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
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[Y] 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

Fujimoto et al studied global changes of mRNA levels, H3K4me3, H3K27me3 in cells infected with retrovirus expressing oncogenic mutant of BRafV600E and compared these changes with their previously reported data in MEF cells infected by congenic Ras. Their data indicate that both oncogenic mutants of Ras and Raf induced cellular senescence and altered mRNA levels of a large number of genes. Among common changed mRNAs, they identified the Bmp2 and Smad6 genes. Their ChiP-Seq data also showed that both of these genes have similar enrichment or reduction of H3K4me3 and H3K27me3, respectively, except that H3K27me3 induction was not found from the Samd6 promoter in RafV600E infected cells. They further showed that knocking-down Bmp2 and overexpressing Samd6 in MEF cells prevented MEF cells from BRafV600E-induced senescence. Their data indicate potential common mechanism underlying cellular senescence induced by mutated Ras and Raf oncogenes. However, this study missed a critical control and a reasonable rationale of why commonly changed, but not differentially expressed, genes were selected for comparison of oncogenic Ras and Raf overexpression. Specific comments. 1. Critical rationale of the current study is missing. Whether there is a difference between replicative cellular senescence and premature

senescence is not described clearly in the introduction. It seems that oncogene(s) can induce both types of senescence. Whether different oncogenes will produce different senescence is unclear. Therefore, it is unclear why the comparison of gene expression in cells induced by different oncogenes should be performed. 2. A critical control is missed from this manuscript, i.e., retroviral vector with empty exogenous gene since viral infection itself may produce cellular reaction(s). 3. Why retroviral vector is used for the overexpression of BRaf and its mutant? What is the infection efficiency of cell population? Whether infected cells must express recombinant gene? Was this expression confirmed? Uninfected cell population may not enter into senescence and thus not undergo alteration of gene expression, therefore resulting in dilution of potential changes of mRNAs in infected cells due to analysis of total RNA from the whole cell population. Should we consider these histone binding as part of chromatin remodeling per se instead of epigenomic change? 4. Why was SA-?-gal analysis only done for cells infected for 7 days? Clearly, this analysis is cytochemical and thus differ from gene expression and ChIP analysis. How can authors explain the difference? 5. Different from na?ve MEF cells, infected cells were screened by G418. Whether this screening will wipe out a special group of cells which are never infected by retroviron, thus producing bias? To the results that cells infected with RafWT showed the "similar" cell number to na?ve cells, considering that G418 removed non-infected cells, it should produce less cell number than na?ve cells. Why did these 2 groups showed similar number of cells? Is this caused by overexpression of Raf? In view of small error bars, statistical examination of cell numbers between RafWT and MEFs should be performed. 6. Why mRNA expression was extracted from cells infected with virus for 5, 7, 10 days, but mRNA expression are examined only for day 5 and 7 and ChIP was only done for day 7 after infection? What are biological repeats of array analysis? 7. In figure 2, 3, 4, why were mRNAs in cells infected with virus expressing wild-type Raf not studied as a control? 8. Writing needs improving. Careful editing should be conducted for the whole manuscript. Examples are listed below. 1) .Conceptual connection at the beginning of introduction is unclear regarding cellular senescence, replicative cellular senescence and premature senescence. 2) Consistence in uses of terms should be greatly taken care of. For examples, replicative cellular senescence and replicative senescence are used; BrafV600E, RafV600E an

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		[Y] No	

COMMENTS TO AUTHORS

In this study, the authors examined the expression changes induced by oncogenic RAF. Previously, the authors have conducted similar study on oncogenic Ras. The main conclusion from this study is that oncogenic RAF and Ras cause similar patterns of gene expression, which is sort of expected, given that oncogenic Ras/RAF function in the same pathway that leads to cellular transformation. I suggest the authors to provide more explanations in the introduction section as why it is important to focus on oncogenic RAF here, given that the work on oncogenic Ras has already been done. A small typo in the first sentence of "Aim" (epigenomics).