Reviewer's report

Title: Functional activation of endogenous p53 by combined, but not individual, p19Arf gene transfer and nutlin-3 drug treatment reduced viability of B16 cells

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Reviewer: Jim Xiao

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In this study, Merkel et al tried to show that either retroviral expression of p19ARF or treatment with nutlin-3, alone, failed to activate p53 in B16 mouse melanoma cells that are p53 wild type and p19ARF null. However, a combination of p19ARF overexpression and nutlin-3 can activate p53 resulting in cell death. This is a problematic study with serious flaws, conceptually and experimentally. Conceptually, nutlin-3 is an extremely potent p53 activator by blocking MDM2-p53 interaction resulting in either cell cycle arrest or apoptosis in p53+ cancer cells whereas the primary function of p19ARF is to inhibit MDM2 ubiquitin E3 ligase activity and stabilizes p53. It is difficult to understand why this group could not to show nutlin-3 activating p53, as many labs in the filed are able to show. With regard to p19ARF, it is possible that the levels of p19ARF overexpression are inadequate. If these are cell type-specific, controls cancer cells should be used to convincingly show that same treatment (p19ARF overexpression or nulin-3) activates p53. Besides, it makes no sense that nutlin-3, a potent p53 stabilizer needs help from p19ARF, which also functions to stabilize p53. Overall, the data as presented were in low quality and the controls were missing or inadequate. This study is clearly premature and the authors need to use proper controls, some of which are list below. It is also evident that the deficiency of scientific writing in English made the reading difficult.

Specific comments:

1. In the abstract. What is meant by: “The p53-responsive pCLPG retroviral vector was used to transfer p189Arf, potentiating interplay of the vector, transgene and endogenous p53 in the B16 cell line”. It is confusing. Does it mean: to study the effects of retroviral-mediated expression of p19Arf on p53 in B16 cells?

2. Fig.1. It should be described in the Materials and Methods, not as a figure.

3. Fig. 2. The data showing that expression of p19Arf failed to activate p53 in B16 cells. There are numbers of reasons. Were expression levels of p19Arf too low? Is p53 truly wild type in the cells used?

4. Fig. 4. It is essential to show that doxorubicin and nutlin-3 activate p53 in B16 cells. Why the levels of p21 protein were extremely low up on drug treatment? There seemed some thing fundamentally wrong for the drug treatment and/or
western blotting for p21.

5. Fig. 5. If p19Arf failed to activate p53 (see #3 above), it is no surprising that it did not inhibit cell growth. Again, were expression levels of p19Arf too low in B16 cells?

6. Fig. 6. It is difficult to understand why nutlin-3 failed to activate p53 in B16 and C6 cells. Did the same batch of nutlin-3 activate p53 in published cancer cells such as U2-OS, SJSA-1 cells?

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Not suitable for publication unless extensively edited

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

**Declaration of competing interests:**

I declare that I have no competing interests.