Reviewer's report

Title: The association of TP53 mutations with the resistance of colorectal carcinoma to the insulin-like growth factor-1 receptor inhibitor picropodophyllin

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Reviewer: Leonard Girnita

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Quan Wang et al., in their article entitled “The association of TP53 mutations with the resistance of colorectal carcinoma to the insulin-like growth factor-1 receptor inhibitor picropodophyllin” have attempted to investigate the responsiveness of colon carcinoma cell lines to IGF-1R inhibition. The study reveals wt p53 as a possible indicator of colon carcinoma sensitivity to IGF-1R targeting by PPP. Overall the study is well designed and easy to follow, however there are two important points not experimentally addressed nor discussed in the study.

Major Compulsory Revisions

As explained in the introduction, PPP was originally discovered as an IGF-1R kinase inhibitor. However, subsequent studies demonstrated the besides inhibiting IGF-1R phosphorylation, PPP also induces IGF-1R downregulation through a mechanism involving the receptor ubiquitination (PMID: 17828296). As long as p53 and IGF-1R share the same ubiquitine ligase MDM2 (PMID: 12821780) and this ligase is under the transcriptional control of the wt p53, the results should be also analyzed and interpreted in this context.

1. One possibility is that different expression of MDM2 among different colon carcinoma cell lines, modify the PPP induced IGF-1R downregulation, thus explaining their higher sensitivity to PPP. To get support for this mechanism I would suggest investigating the expression status of MDM2 as well as the kinetic of IGF-1R degradation (0, 12 and 24 h) following treatment with IGF-1, PPP and PPP+IGF-1. Increased (or decreased through receptor recycling) receptor degradation in wtp53 versus mtp53 cell lines might provide clues regarding the mechanism of their sensitivity.

2. An apparent paradox is presented in Fig. 2A for the CACO-2 cell line: although the kinase activity of the IGF-1R is inhibited by PPP (as demonstrated by receptor phosphorylation) the AKT and ERK are (hyper) phosphorylated. Thus, another possibility that should be taken in consideration to explain the limited response to PPP in cells expressing mtp53 is the “biased” signaling activation by PPP. As described above, what differentiate PPP from other IGF-1R tyrosine kinase inhibitors is receptor downregulation. On the other hand the IGF-1R downregulation is a common feature for anti-IGF-1R targeting antibodies. It has been shown that PPP induced receptor downregulation activates ERK signaling (PMID: 18070930). Likewise, in the case of anti-IGF-1R antibodies, it has been demonstrated that “biased” IGF-1R biased signaling is responsible for resistance
to anti-IGF-1R therapy (PMID: 23188799). Therefore I would suggest testing the kinetic of ERK phosphorylation following short time (0 to 60 min) stimulation with PPP only in serum-free media.

Nevertheless the possible resistance mechanisms to PPP as well as demonstrated signaling activation with low IGF-1R phosphorylation in mtp53 versus wtp53 mtp53 should be addressed in the discussion.

Minor Essential Revisions

There are some spelling errors or missing words. For the PPP description I would suggest using the term “IGF-1R inhibitor” instead “IGF-1R kinase specific inhibitor”

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**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.