Author's response to reviews

Title: Deficiency of G1 regulators P53, P21Cip1 and/or pRB decreases hepatocyte sensitivity to TGFbeta cell cycle arrest

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Research article
Deficiency of G1 regulators P53, P21Cip1 and/or pRB decreases hepatocyte sensitivity to TGFbeta cell cycle arrest

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Thanks for allowing us to review our manuscript. The points suggested by the referees’ report have been addressed. Please find below our response to the referees, with the details of the revisions in the manuscript.

We hope you will find these corrections satisfactory. If you need any more information, please do not hesitate to contact me.

Best regards

Dr S Prost

Reviewer 1

We thank the referee for the very positive review.

Reviewer 2

We agree that the involvements of Rb, p53 and p21 in the regulation of TGF beta induced inhibition of proliferation have already been shown and we have added a sentence to emphasise this in the introduction with appropriate references (page 5 end of first paragraph). However, as p53 activates p21 transcription, and p21 is a regulator of Rb phosphorylation, it is somewhat difficult to define whether p53 and p21 may affect the regulation of proliferation by TGFbeta independently of Rb. This is an important question in various systems, and particularly here, in chronic liver diseases where various hepatitis proteins can inhibit Rb, p21 or p53. We have added a sentence to clarify this point (page 5, last paragraph).

In the present study, we used a combination of deletions of these three genes to convincingly show that indeed in the absence of Rb, p53 but not p21 affects hepatocyte responses to TGFbeta. This means that in hepatocytes deficient for Rb but that maintain functional p53, a certain level of regulation of proliferation by TGF beta remains. However, loss of both proteins’ function (inhibition by viral proteins for example) would have further detrimental effect on this regulation.

To our knowledge, our present study using combinations of targeted deficiency in 3 mains regulators of E2F activity (through Rb) response to TGF beta in combination has not been done by others, and here allows us to decipher the individual roles of each protein, which are otherwise difficult to discriminate because of their interrelations.

The referee points out a “discrepancy” between the higher level of CDKI expression and the level of CMYC activity that are both higher in Rb null cells than wild type cells. We believe that there is no such discrepancy but the referee’s comment has highlighted a missing point in our results section that we have now added. We have also changed the text to clarify this point: We agree that it is well known that CMYC is a negative regulator of CDKI expression. However this is not in contradiction with our data. Forty-eight hours after plating, CMYC expression was not significantly different in wild type and Rb null cells (this is now stated in the text page 10 in “Rb is central to TGF beta –induced inhibition of proliferation” and continuing page 11). It is at this point of the cell cycle that we find that the level of CDKI are increased in Rb null cells. We can only speculate why this is happening, but there is evidence
that E2F activity can regulate CDKI [1]. After Rb deletion, E2F activity increases rapidly and may activate CDKI transcription before the system reaches an equilibrium. Seventy-two hours after plating however, the level of CDKIs expression returned to the same level as observed in wild type cells, and the increased level of cmyc activity may indeed, as suggested by the referee, be involved. We have changed the text (results page 10) to highlight this and thank the referee for pointing it out.

Reference List