Author's response to reviews

Title: The pro-apoptotic K-Ras 4A proto-oncoprotein does not affect tumorigenesis in the ApcMin/+ mouse small intestine

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MS: 1922922811171449: Response to reviewers’ comments.

We are pleased to note that both reviewers feel that our findings are important to those with closely related research interests and that our manuscript should be accepted after minor essential revisions. Both reviewers ask for further histopathological characterisation of the tumours, with evaluation of proliferation and apoptosis, and one reviewer asks for measurement of the K-ras 4A/4B transcript ratio in Min mouse tumours.

We have performed all the additional work requested. Consistent with our observations on tumour number and size, there is no effect of K-ras 4A on tumour histopathology, tumours in both mouse genotypes being exclusively adenomas of very similar, mild to moderate, dysplasia, with no evidence of invasive carcinoma. We have evaluated proliferation and apoptosis by the gold standard methods of scoring mitotic and apoptotic figures in haematoxylin and eosin sections rather than the surrogate immunohistochemical methods suggested by one reviewer. Our co-author Mark Arends is particularly well qualified to do this, being an experienced histopathologist who has published extensively on apoptosis since his early collaborative work with Andrew Wyllie. There is no significant difference in apoptotic or mitotic counts between the genotypes, consistent with our other findings. These results are now included in the revised text at the end of the second paragraph of the Results section, and the methods detailed under the subheading Histology of the Methods section.

We have compared transcript ratios in tumours of the small intestine and in normal small intestine in Min mice, using the same methods as for the data in Figure 1. These data are presented in an additional figure (the new Figure 2; the old Figure 2 is now renumbered as Figure 3). Although both Figure 1 and Figure 2 now report transcript ratios in normal Min mouse small intestine, we have kept them separate rather than combining them as each figure represents an internally controlled experiment: the data in Figure 1 are from young mice age- and sex-matched to wild type mice, while those in Figure 2 are from the same ageing mice as the tumours. As in human colorectal cancers, the 4A/4B transcript ratio is reduced, but in the Min tumours this is accounted for by an increase in 4B levels without appreciable change in 4A. This is entirely consistent with our conclusion that the 4A isoform does not affect tumorigenesis in the Min mouse small intestine: however, as emphasised in the text, it does not render the data of Table 2 and Figure 3 redundant and both are necessary for our conclusions.

As a result of these revisions we have one additional author, Rachel A Ridgway.

We trust that the revised manuscript is satisfactory and look forward to the editor’s decision.