Author's response to reviews

Title: Regional differences in prostaglandin E2 metabolism in human colorectal cancer liver metastases

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Response to Reviewers’ comments

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Reviewer #1. Christos Paraskeva

1. More information on the molecular background of LIM 1863 cell (eg basal COX-2, 15-PGDH and TGF beta receptor expression/SMAD) would be useful. Where 15-PGDH is epigenetically silenced it may not be so readily affected by tumour microenvironment.

We are not aware of any previous reports characterising eicosanoid metabolism by LIM1863 human CRC cells. Our studies have shown that, although these cells express COX-2, PGE₂ is not detectable in cell-conditioned medium. Early reports described down-regulation of the type II TGFβ receptor in LIM1863 cells after TGFβ treatment. We have added a statement that LIM1863 cells express COX-2.

2. The comment (first page of discussion, first paragraph) about tacitly accepted that the increase in PGE₂ content…occurs uniformly… I think this comment needs to be modified as I am not sure this is correct. Given that the tumour microenvironment is known to affect the expression of key genes and the tumour microenvironment is itself heterogeneous I am not sure this comment is strictly correct. Why was there no significant heterogeneity of COX-2 protein expression if hypoxia id heterogeneous and regulates COX-2 expression?

We have removed this statement from the text in order to avoid confusion. Removal does not alter the sense of the opening summary paragraph of the Discussion.

3. Is there a reference or evidence for the metastases being more hypoxic as this is an important point?

We are not aware of any direct comparison between primary CRC and secondary CRC liver metastases concerning hypoxia markers.

4. A brief reference to COX-1 derived Prostaglandins being potentially important could be included in the manuscript.

Cox-1 has been implicated in intestinal tumorigenesis in the Min mouse model of the early stages of colorectal carcinogenesis, which are not necessarily relevant to advanced CRC. Therefore, we have not added a statement about the possible role of COX-1-derived eicosanoids, particularly as this would increase the length of the Introduction.

5. It could be worth considering that the heterogeneity of 15-PGDH might at least in part, be related to recent studies showing that beta catenin can suppress 15-PGDH levels (Smartt et al Gut, 2012) and that hypoxia can in some conditions block beta catenin activity (Kaidi et al NCB, 2007).

This line of thinking has been added to the Discussion (page 16) and the two references have been included.

6. In the discussion, the use of the word “functional” with protein is a bit confusing, might be better to leave “functional “out and keep in protein on its own.
The term ‘functional’ has been removed from the Discussion first paragraph

Reviewer #2. Peter Kuppen

*It is for instance* not clear why liver metastases have been chosen, why not primary tumors? This work led directly from our previous projects (*Gastroenterology* 2003;125:716 and *Gut* 2006;55:1058) on the effect of the selective COX-2 inhibitor rofecoxib on CRC liver metastases (previously believed to be a promising therapeutic strategy). We therefore focussed mainly on CRC liver metastases. We do present some data comparing CRC liver metastases to paired primary tumours.

*Furthermore, tumors* (heterogeneous) are compared with cell lines (expected to be homogeneous). This should be clarified. It would probably be much better if a comparison among primary and metastatic tumors would be made. We did compare paired primary CRC and CRC liver metastasis tissue from the same patients (page 9 and Supplementary Figure 2) and demonstrated that primary CRCs did not display differential 15-PGDH levels in the centre and periphery of tumours.

*Finally, the authors are discussing therapy, but this is beyond the context of the paper and not supported by any data.* We have removed the last paragraph of the Discussion in which we speculate on the therapeutic implications of our findings.