Author's response to reviews

Title: In vitro and in vivo MMP gene expression localisation by In Situ-RT-PCR in cell culture and paraffin embedded human breast cancer cell line xenografts

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Author's response to reviews: see over
Rebuttal Comments:

Haupt et al; Manuscript 984781574415329 - In vitro and in vivo MMP gene expression localisation by In Situ-RT-PCR in cell culture and paraffin embedded human breast cancer cell line xenografts.

Response to Reviewer #1:

1.1 We thank the reviewer for their comments. As requested the Discussion section has been tightened, including updating some general MMP information.

1.2 In addition the Reviewer asked that the quality of the images attempt to be improved. We apologise for this, the quality of the images has been affected during file transfer. We have returned to the original images and have further increased the pixilation ratio of the images. We think there is an improvement in the clarity of the images and hope that the Reviewer is happy with the improvement in the image quality.

Response to Reviewer #2:

2.1 We thank Reviewer #2 for their feedback. To objective of the study has been clarified in the Introduction Page 5, Line 2 as outlined below.

“Thus the objective of this study was to utilise the unique IS-RT-PCR methodology to examine the localised gene expression of members of the MMP family of proteases implicated in breast cancer. Our findings here.....”

We hope that this clarification has added more significance to the Conclusion in the Abstract on Page 2, Line 11 as detailed below:

“Conclusion: These results demonstrate the applicability of IS-RT-PCR for the examination of MMP gene expression in vitro and in vivo. In addition, the data highlight the contribution of the stroma and the complexity of the role of MMPs in the sromal-epithelial interactions within breast carcinoma.”

Response to Reviewer #3:

3.1 Reviewer #3 has highlighted some interesting literature missing from the manuscript that was initially omitted. The literature review in the manuscript has been updated where applicable, including the inclusion of our other recent data examining relative basal levels of MT1-MMP gene expression between MCF-7 and MDA-MB-231 cells, and data highlighting the in vitro cell density effects on MMP gene expression. The manuscript has been amended as outlined below, Page 3, Line 27, as well as in several paragraphs throughout the Discussion on Pages 12 to 16.
“MT1-MMP is one of the membrane bound MMPs. In vivo, MT1-MMP expression has been localised to the stroma surrounding breast tumours [11, 12], whilst in vitro, our recent data confirms previous studies where basal levels of MT1-MMP have been shown to be higher in the more invasive MDA-MB-231 cells as compared to the less invasive MCF-7 cells [13, 14].”

3.2 Reviewer #3 has correctly pointed out the increased number of MMP species reported to date. The manuscript has been changed on Page 3, Line 8 as outlined below.

“Currently, 28 human MMPs have been identified and classified according to both their substrate specificities and structural similarities. There are four major subgroups: i) interstitial collagenases; ii) gelatinases; iii) stromelysins; and iv) the membrane-type (MT) -MMPs [3, 4].”

3.3 The typographical error noted by Reviewer #3 on Page 10, Line 12 of the manuscript has been corrected.

3.4 Again, Reviewer #3 highlights our more recent data, in Lafleur et al, discussing the basal gene expression levels of MT1-MMP in MCF-7 and MDA-MB-231 cells. This data has been highlighted as outlined in Response 3.1 and referred to accordingly.

3.5 As requested by both Reviewer #1 and Reviewer #3, we have updated the reference list where appropriate. Several additional references have been included with recent data on MMP gene expression in HBC cell lines and density effects observed in vitro and have been discussed in context in Pages 12-16. The Bibliography has increased to 68.