Author's response to reviews

Title: Heparan sulfate proteoglycans undergo differential expression alterations in right sided colorectal cancer, depending on their metastatic character

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Author's response to reviews: see over
Dear Editors:

Please find enclosed a revised version of our manuscript “Heparan sulfate proteoglycans undergo differential expression alterations in right sided colorectal cancer, depending on their metastatic character”, in which we have included the modifications suggested by the referees (highlighted in red in the text). We have also highlighted in red in the methods section that written informed consent was obtained from patients. In addition, attached to this letter we include a letter in which we address the concerns raised by the referees. We hope that this revised version fits the requirements for publication as an Article in BMC Cancer.

Yours faithfully,

Dr. Luis M. Quirós
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Response to reviewer 1:

Dear Dr. Tzanakakis:

Many thanks for considering our article ready for publication.

Response to reviewer 3:

Dear Dr. Gharbaran:

Thank you very much for your thorough review of the article. We have included all your suggestions in the text, and they appear highlighted in red.
Response to reviewer 2:

Dear Dr. Filmus:

We do agree with you on some of your opinions. Certainly, it would be interesting to analyse the set of HSPGs as final products, that is, detecting the core proteins, as well as other aspects such as their location, shedding and other interesting aspects. Similarly, the structural analysis of glycosaminoglycan chains, including aspects such as chain length, the disaccharide composition or their domain structure would be of interest. However, this would not be feasible in an approach that encompasses as many different molecules as are analysed in this study and furthermore, in some respects, it would be difficult to carry out technically.

That said, we do believe that our approach is of value because it provides an overview of the changes in the molecules mentioned above, something which is scarce in the literature (usually more focused on specific molecules), and can provide a basis for more specialized further studies.

While acknowledging the basis of the objections you raise, we do not believe that they invalidate the results presented in the article for the reasons set out below:

Concerning your statement about the use of qPCR in homogenized tissues, you are no doubt aware that, reflecting its dominant function in absorption, the right colon displays a higher enterocyte to goblet cell ratio (roughly 5:1) compared to the left colon (between 4:1 and 3:1). The other cell types you mention have an even lower proportion (Histology for Pathologists, by Stacey E. Mill). In fact, the methodology we employ has been widely used in the literature with different tissues.

In addition, you mention the discrepancy between the levels of transcription and protein expression determined by immunohistochemistry, as in the case of syndecan-1. As indicated in the text, our results were confirmed using CISH, and are in perfect agreement with previous data from other authors who are cited in the literature (references 35-39), and post-transcriptional regulation of syndecan-1 expression has been indicated previously (references 39, 41). However, syndecan-1 is an exception in this study, since most genes analysed showed a good correlation between transcription and protein expression values. This has been also described in some previous works by other authors, who have analysed the relationship between the levels of gene transcription and protein expression in tumours. As such, in studies of lung adenocarcinoma it has been described that the majority of genes analysed did not differ in protein/mRNA correlation, indicating a similar regulatory relationship between mRNA and protein (Chen et al, 2002; Mol Cell Proteomics 1: 304-13); Similarly, another example would be in studies of various tumours, such as bladder cancer, that have shown a good correlation between transcript alterations and protein levels, with few exceptions (Ørntoft TF et al, 2002; Mol Cell Proteomics 1: 37-45).