Author's response to reviews

Title: Podocalyxin is a marker of poor prognosis in colorectal cancer

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Author's response to reviews: see over
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Dear Editor-in-chief,

Enclosed please find our two revised manuscripts “Podocalyxin is a marker of poor prognosis in colorectal cancer” and “A comparative study of two PODXL antibodies in 840 colorectal cancer patients”, both under MS: 1596918575116611.

We thank the referees for their valuable comments. We have responded to all their comments and have made corresponding changes to the manuscripts. We sincerely hope that our revised manuscripts will be suitable for publication in BMC Cancer, and that they will be published as two separate parallel papers, in the same issue of your journal as suggested by the referee.

Yours sincerely,

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Enclosed:
-Response to the referees comments
-Revised manuscript: Podocalyxin is a marker of poor prognosis in colorectal cancer
  -version with the changes highlighted
-Revised manuscript: “A comparative study of two PODXL antibodies in 840 colorectal cancer patients”
  -version with the changes highlighted
Answers to referees comments

Referee 1

Referee: It will be helpful for the audience if the authors could elaborate further on the cytoplasmic localization of PODXL in the cancerous specimens. I would expect that PODXL as a potential stem cell marker is present on the cell membrane, too.

We: By mAb HES9 the expression of PODXL was cytoplasmic, evenly distributed, and the staining pattern was often granular. No nuclear positivity was seen. Membranous positivity was seen only in cells with strong cytoplasmic staining. Similar change in expression from membranous to cytoplasmic is also seen for instance in some Toll-like-receptor (TLR) stainings. We made corresponding changes to the manuscript.
Referee 2

Referee: The authors report that the staining patterns of both reagents are different. In the discussion they hypothesize that different function or maturation might be responsible. However, they do not refer to information that is readily available in the internet on the differences in molecular weight of podocalyxin. Roughly there is a substance with a molecular weight of 55-60Kda and one of 165Kda. I would appreciate if the authors could include such information and if known the reactivity of the reagents used with one or both of these substances.

We: Of ten PODXL splice variants, four are protein coding and their molecular mass varies between 55 and 59kDA (The Human Protein Atlas). Of the four protein coding PODXL splice variants, the epitope sequence of the pAb matches three with 100% (PODXL 001, 005, and 201, The Human Protein Atlas/Atlas Antibodies). The fourth splice variant matches with 87% (PODXL 202). The epitope sequence of the mAb HES9 matches all splice variants with 100%.

After posttranslational processing, mainly glycosylation, the molecular weight of PODXL reaches 165kDA. (Kershaw, 1997). Both antibodies studied mainly react with the peptide backbone.

We made corresponding changes to the manuscript.