Author's response to reviews

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

Authors:

Tuomas Kaprio (tuomas.kaprio@helsinki.fi)
Christian Fermér (christian.fermer@fdab.com)
Jaana Hagström (jaana.hagstrom@hus.fi)
Harri Mustonen (harri.mustonen@helsinki.fi)
Camilla Böckelman (camilla.bockelman@helsinki.fi)
Olle Nilsson Nilsson (olle.nilsson@fdab.com)
Caj Haglund (caj.haglund@hus.fi)

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

We thank for valuable referee comments on our two manuscripts on podocalyxin in colorectal cancer and the editor for accepting our results as two separate papers. Our first manuscript: *Podocalyxin is a marker of poor prognosis in colorectal cancer* under MS: 1596918575116611. The second paper “A comparative study of two PODXL antibodies in 840 colorectal cancer patients” was submitted and reviewed under the same MS number. We now submitted the second manuscript and our comments to referees as a separate submission according to your instructions.

Enclosed please find our re-revised manuscript ”*Podocalyxin is a marker of poor prognosis in colorectal cancer*” under MS: 1596918575116611. We have highlighted the changes according to editor’s comments.

We sincerely hope that the editor find our revision appropriate and hope that the re-revised manuscript is now suitable for publication in BMC cancer.

Yours sincerely,

Tuomas Kaprio, M.D.   Caj Haglund, M.D., Ph.D.
FIN-00290 HUS, Helsinki, FINLAND   FIN-00290 HUS, Helsinki, FINLAND
Phone: +358 505910353   Phone:+358 9 47172427
E-mail: tuomas.kaprio@helsinki.fi  Email: caj.haglund@hus.fi

Enclosed:
-Re-revised manuscript: *Podocalyxin is a marker of poor prognosis in colorectal cancer*
Answer to editors comment

Editor

Authors could elaborate further on the cytoplasmic localization of PODXL in the cancerous specimens

Authors: This phenomenon that the editor has commented on is really interesting. For several biomarkers we and others have seen that membrane bound proteins in normal cells are differently in cancerous tissues. Often the staining may be cytoplasmic and in some cases membranous staining is less prominent. One example of this is TLR which we recently reported (Hagström J, Heikkilä A, Siironen P, Louhimo J, Heiskanen I, Mäenpää H, Arola J, Haglund C: TLR-4 expression and decrease in chronic inflammation: indicators of aggressive follicular thyroid carcinoma. J Clin Pathol 2012, 65:333–338.).

It is unclear whether the cytoplasmic expression in cancer tissues is due to difference of PODXL function in cancer or that the immunohistochemical staining recognizes different PODXL splice variants in cancer compared to benign tissue.

We included corresponding thought and comments to discussion.

Previous 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.