

31th May, 2016 Budapest

Dear Editor,

We are indebted to the reviewer for the further suggestions to improve our manuscript (Manuscript No.: 25915), entitled "Increased duodenal expression of miR-146a and -155 in pediatric Crohn's disease". We have carefully studied and appreciated the criticism and comments of the Referee. The paper was rewritten to answer the raised questions and suggestions. Additional data were included and changes were highlighted with yellow in the manuscript. Thank you very much for your suggestions. A medical translator/linguist has reviewed the manuscript, and we corrected the paper linguistically according to her suggestions.

I hope our manuscript is now in an acceptable form for publication.

Yours sincerely,

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Answers to the comments of Reviewer 1:

1) In Materials and Methods authors should specify the protocol used to isolate primary cells, or indicate a reference.

Thank you for your comment. We completed the description of the isolation protocol of primary cells in Materials and Methods, as highlighted with yellow on page 6.

2) They should also explain why they used tissue obtained from healthy controls, and not subjects with CD.

We fully agree with the reviewer, that it would be interesting to explore the role of TGF- β on the expression of miRs in primary duodenal fibroblast cells derived from patients suffering from Crohn's disease (CD). However, in our present study we aimed to focus primarily on the basic effects of TGF- β on duodenal cells and not on a complex relation with other inflammatory cytokines and growth factors present in the gastrointestinal tract of CD patients. Therefore, according to our above aims we performed our experiments on the samples of healthy controls and not on subjects with CD.

3) Authors should also clarify whether the diseased and healthy tissues were obtained from intestinal biopsies derived, at least in some cases, from the same patient. From the data of Table 1, it seems that in many cases biopsies were derived from different patients, this requires a comment.

We are grateful for the remark. At pediatric endoscopy it may be difficult to collect accurate biopsies from both ulcerated and healthy regions of the same CD patient, due to the fact that pediatric CD is more often extensive disease (L3 and L4 in the same patient) leaving only a limited space to non-inflamed region. On the other hand, in cases of CD with colonic- or distal ileocolonic involvement the upper GI tract is often not affected. To reach considerable number of tissue samples we had to include biopsies from different patients (we added this information to the manuscript highlighted with yellow on page 6).

4) Authors should add the RNA extraction protocol, or a reference.

We added the extraction information to the RNA isolation protocol, as highlighted with yellow on page 7 in the manuscript.

5) In the previous paper, mentioned above, the role of miRNAs has been studied using, both in immortalized and primary cells, TNF alfa as inflammatory stimulus. It would be interesting to do the same on duodenal cells, and possibly the effect of TGF beta could be evaluated on this pattern of inflammation.

We agree with the reviewer that it would be interesting to investigate the effect of TNF- α on immortalized small intestinal epithelial and primary duodenal fibroblast cells too. Similarly to our previous study examining the link between IBD-related core cytokine TNF- α and miR-146a, -155 and -122 in the colon of children with IBD using immortalized colonic cells, we plan to perform these measurements also on duodenal cells in a future study. Moreover, we consider to evaluate the effect of TGF- β on this inflammatory pattern as suggested by the reviewer.

6) Tables 2 and 3 can be removed. This is not a review, and the most relevant articles can be discussed in the text.

According to the reviewer's suggestions we removed table 2. and 3 and discussed the related information in the manuscript text, highlighted with yellow on page 10 and 11.

7) Finally, English should be reviewed, for example the term primer referred to duodenal cells is not clear.

We are grateful for the remark. We reviewed our English, and the term "primer" that is referred to the "primary" duodenal fibroblast cells was corrected throughout the whole manuscript. In addition, a native speaking expert helped to improve the English in the paper.