April 23, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format.
Title: Association of HGFR/CDX2 coexpression with mucosal regeneration in active ulcerative colitis
Author: Ferenc Sipos, Miklós Constantinovits, Gábor Valcz, Zsolt Tulassay, Györgyi Műzes
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 15654

The manuscript has been improved according to the suggestions of reviewers:

Dear Reviewer #02999941,

First, we thank you for your extensive review and useful suggestions.
Here are our answers to your question:

Major points:
1. Participants of the control group included those, who underwent colonoscopy for screening CRC. Routine biopsies were taken also for the study purposes. None of the involved persons have been suffering from any other documented inflammatory or tumorous disease conditions.
Only biological samples of patients with histologically proven active ulcerative colitis were used for study purposes. Therefore no Crohn's disease subjects were involved. Determination of UC activity was based on Mayo scoring system.

2. Only patients with histologically proven active ulcerative colitis were involved to the study, and the obtained data were compared with those of healthy controls.
A so-called "dose-response" trend could not be determined, since no mild and moderately active UC patients were involved.

Minor points:
1. Up to present there are no available data regarding the hypothetic effects of colonoscopic preparation on number and characteristics of colonic stem cells. Nonetheless, without a bowel preparation biopsy samples cannot be collected.

2. In case of 90% of the active UC patients left-sided colitic biopsies were performed, especially because of technical aspects, and only 10 % of patients underwent right-sided sample taking. Since the total number of the right-sided samples was quite low, statistically it is not relevant and accepted to compare such gene expressions.

3. Chronic fibrosis and tumorigenesis can both result from a prolonged and exacerbated healing response that could stem from chronic injury. In fact, chronic fibrosis constitutes a direct cancer predisposition [Schafer M, Werner S. Cancer as an overhealing wound: an old hypothesis revisited. Nat Rev Mol Cell Biol 9: 628–638, 2008].

We hope that are answers are acceptable for you.
Thank you again for your valued comments.

Dear Reviewer #03000422

First of all, we thank you for your crtitical review and useful suggestions.
Here are our answers to your question:

1. In UC, the exact mechanism of the recruitment of HGFR/CDX2 double positive cells from blood to LAs is largely unknown. In general, HGF augments cell migration, scattering and proliferation of many different cell types, predominantly of epithelial cells. In UC elevated serum HGF level was found. Additionally, recent results indicate that the HFG/HGFR-Cmet system is deeply involved in the repair process of the inflamed mucosa in UC. The increased systemic HGF may mobilize bone marrow or tissue resident (colonic) stem cells to colonize the damaged target tissues. Production of HGF and expression of HGFR/C-met are especially related to a stromal, mesenchymal cell population. Expression of C-met/HGFR by stromal cell may sugges an autocrine stimulation of stromal cells by HGF. The classic concept of tissue repair means that inflammatory cells entered the damaged tissu and signal resident signal resident tissue-specific progenitor cells (eg: parenchymal cells, fibroblasts) for mitosis. Furthermore, circulating immature stem cells could also participate in regeneration of many different tissues. CDX2 is essential to intestinal homeostasis and regulate the balance and differentiation of epithelial cells. The HGFR/CDX2 double cell positivity in itself indicate the existence of MET.

2. The exact origin of these double positive cells is largely unknown, and remains to be determined in the near future. Generally, CD133+ cell fraction contains more mesenchymal stem cells. Several studies proved that mesenchymal cells could express a lot of gene products prior to their differentiation. MSCs are highly plastic and portray a variety of phenotypes. MSCs were also shown to be able to differentiate directly into (CDX2 +) epithelial cells. Lgr5+ stem cells are intestinal stem cells, but some of them co-express CD133.
CDX2 is essential to intestinal homeostasis and regulate the balance and differentiation of epithelial cells. Musashi1 is also expressed both by intestinal stem cells.
Lgr5 along with CD133 are typical Wnt target genes, while Musashi1 promotes Notch signaling.
Both Wnt and Notch pathways are known to regulate stem cell behaviour. In addition, Musashi1 + cells might also represent circulating smooth muscle cell precursors. The cellular plasticity (including the phenotypic markers of stem-like cells) may represent an adaptive mechanism after injuries, like intestinal inflammation for the self-preservation of the epithelial layer.

3. Based on the results of our previous chromosomal chimerism experiments we have already proved that part of epithelial stem cells are originated from a local stem cell pool (e.g. the CD133/CDX2 population), while another part of them is of marrow origin. [Valcz G et al. Lymphoid aggregates may contribute to the migration and epithelial commitment of bone marrow-derived cells in colonic mucosa. J Clin Pathol. 2011 Sep;64(9):771-5. doi: 10.1136/jclinpath-2011-200022.]
As the marrow aspiration is a painful diagnostic procedure, it is not really possible to get the consent of UC patients to undergo such an examination just for study purposes.

We hope that are answers are acceptable for you.
Thank you again for your useful comments and suggestions.

Dear Reviewer # 00044509

Above all, thank you for reviewing and commenting our manuscript.
Here are our answers to your question:

1. At present we are not familiar whether our findings are specific for UC or not, since only active UC patients were involved to the study.

2. CD133 represents another target gene for the regenerative Wnt signaling pathways. CD133 is of importance in intestinal regeneration and could decrease inflammation. CD133+ cells are considered as a population of non-committed early progenitors capable of self-renewing and differentiating into blood cells and other cell types. It is also supposed that CD133+ cell fraction contains more mesenchymal stem cells.
Several studies proved that mesenchymal cells could express a variety of gene products prior to their differentiation. Although it is clear that the intestinal epithelium responses to inflammation and mucosal injury by initiating a regenative response, the specific inflammatory effects on the turnover of epithelial stem or progenitor cells, and the way in which the inflammatory milieu may perturb epithelial differentiation and function, remain obscure.
Recent results support the view that the inappropriate expressions of both HGF and HGFR genes may predispose to the development of CRC in patinets with UC.
There are data indicating that CD133 could also be viewed as a cancer stem cell marker. However, in CRC both CD133+ and CD133- cells may possess a tumorigenic potential. The reciprocal interactions between stem cells and their niche also influence stem cell behaviour.

3. The introduction has been shortened.

We hope that are answers are acceptable for you.
Thank you again for your valued comments.

We also thank to Reviewer #03008962 and Reviwer #00055213 for supporting the publication of our manuscript.

The format of the manuscript also has been improved.
All changes are highlighted by yellow colour in the text.
We do hope that our answers are acceptable for the Editors of WJG.
Thank you again for considering the possible publication of our manuscript in World Journal of Gastroenterology.

Sincerely yours,

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June 8, 2015

Dear Editor-in-Chief,

Please find enclosed the revised version of our manuscript in Word format.
Title: Association of hepatocyte-derived growth factor receptor/caudal type homeobox 2 co-expression with mucosal regeneration in active ulcerative colitis
Author: Ferenc Sipos, Miklós Constantinovits, Gábor Valcz, Zsolt Tulassay, Györgyi Műzes
Name of Journal: World Journal of Gastroenterology
ESPS Manuscript NO: 15654

The manuscript has been improved according to your useful suggestions.

Here are our answers to your question:

Regarding your question about the mechanism of the chemoattraction of bone-marrow derived cells to the lamina propria one cannot exclude the possibility that stromal cell-derived factor 1 (SDF1) or VEGF have a role in this function. In our study, we did not study these factors. However, HGF alone is also able to display a chemoattractive function since it has been reported that HGF level of the inflamed colonic mucosa is elevated as compared to that of normal samples. The HGF/HGFR system may play a chemoattractant system as well.

The format of the manuscript also has been improved.
English language has been polished.
All changes are highlighted by green colour in the text.
We do hope that our answers are acceptable for the Editors of WJG.
Thank you again for considering the possible publication of our manuscript in World Journal of Gastroenterology.

Sincerely yours,

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