Dear Dr. Qi,
We are grateful for the constructive critiques our manuscript (26996) received and feel that we were able to respond to the questions or suggestions with changes that improved our work. I hope that you will find the revised version of the manuscript acceptable for publication in the World Journal of Gastroenterology.
Attached is a listing of the detailed responses to each of the reviewers´ #1 and #2 comments. The reviewer #3 liked the paper as such and recommended publication without further changes.

Response letter

1. Why was the Expression only rated by either intensity or extensity and not via h-score (intensity x extensity)? This would be a more standard approach and should be shown.

- There are several ways to present immunohistochemical scoring data. According to the reviewer´s suggestion we tested the results using the index of (intensity x extent). In Kaplan-Meier analysis, CA II showed a statistically significant result with the p-value of 0.006. CA XII showed a nearly significant p-value of 0.052. These results support our previous findings. We have separately analyzed both intensity and extent results, because they reflect different phenomena. The staining extent indicates the number of positive cells, whereas the staining intensity tells more about the changes of expression within each cell.

2. Were the statistical results corrected for multiple testing?

- No, they were not. We have changed the misleading sentence in the methods.
The new sentence reads (page 12, lines 227-229): "When analyzing the expression of each CA enzyme, level 0 was considered the baseline, and the announced hazard ratio rises exponentially every time the scoring value rises one unit."

3. While the number of cases is adequate, it is unclear if also colorectal adenomas were included.

- In our article, we explain that we studied only colorectal carcinomas, and therefore no colorectal adenomas were included.
Page 6, lines 75-76: "Our aim was to investigate expression of four alpha-carbonic anhydrases (CAs) in colorectal carcinomas (CRC) and compare the results with patients' survival."

4. The collection period of the samples was very long! What is the influence of changed treatment regimens (e.g. radiation on rectal cancer) on the observed survival times? This could be a strong confounder.

- This material included only very few patients who had had cytostatic therapy, and some rectum carcinoma patients had had preoperative radiation therapy. Due to the large number of patients in this cohort, we strongly believe that changes in the treatment regimens had no or only minor effect on the survival times.

5. Does the protein expression of CA also correlate with its enzymatic activitiy?

- Each carbonic anhydrase isozyme has its own specific enzymatic activity depending on the molecular structure. When the enzyme expression increases in the tissue, the number of enzyme molecules consequently increases, which in turn, leads to higher enzymatic activity per milligram of tissue. The enzyme activity remains exactly same per enzyme molecule.

6. The authors need to be careful when using the term "prognostic" related to treatment outcome. This is rather a predictive marker in this context.
- We agree with the reviewer that the term "predictive" would be appropriate in this context. Therefore, we have replaced the term "prognostic" with "predictive" when we discuss treatment outcome.

7. The term "gender" should be replaced by "sex" in table 1.
- We modified the table according to the reviewer´s suggestion.

8. There seems to be a bias towards rectal cancer in the study population, which needs to be discussed.
- Our study material includes samples from a consecutive series of colorectal cancer patients treated in our university hospital. This is why we might have more rectal cancer patients in this cohort than expected. Some patients with colon cancer were treated in other hospitals and were thus not included.

9. Does the term "differentation" relate to grading?
- Yes, it does. We added this information to the Table 1 and to the text (page 11, lines 213-215):
"Because neither Dukes classification nor histological differentiation grade status followed the Cox model assumption, stratified analyses for Dukes stage and differentiation grade were used."

10. In Table 2, the values for extent do not seem to fit with the examples shown in Fig 1
- For the Figure 1 we preferred samples that showed clear staining, not necessarily those that showed medium or weaker staining. It should be noticed that even if the mean value of the staining extent was 1.6, for example, there were samples where all tumor tissue was strongly stained and therefore the extent was graded as 3, as well samples where the staining was absent with the score 0.

11. A scale bar is missing in Figure 1
- We have added the scale bars to each panel of Figure 1.

12. Page 3 line 70: please define "extent".
- Extent in this particular sentence means extent of staining. It is defined in detail in the Immunohistochemical analysis –section, page 11, lines 198-201. "The extent of the staining was scored as 1 when 1-10% of the cells stained, 2 when 11-50% of the cells

stained, and 3 when 51-100% of the cells stained. A negative score (0) was given to tissue sections that had no evidence of specific immunostaining."

13. Were all cases of colorectal cancer sporadic, or have other subsets (IBD-related, familial adenomatous polyposis or other inherited CRC) been included?
- This study material includes specimens from consecutive colorectal cancer patients treated in Helsinki University Hospital. Based on the typical prevalence of hereditary cancer, this material probably includes a few such cases. Unfortunately, this information had not been systematically registered during the collection period, and was thus omitted from the paper.

14. Despite immunohistochemistry for CA in normal colon tissue has been performed, the authors did not report the intensity of the staining in this control group. It is important to compare the expression of CA in CRC vs normal colon.
- The normal colon staining images were taken from the same tissue array samples as CRC images. Many of the CRC samples contained normal tissue in addition to the pathological lesions. However, normal colon tissue was not graded for the staining indices, because the areas adjacent to the actual tumor may not always represent the best and most accurate region for quantification of staining results. Another reason was that CA immunostaining has been previously well documented in the normal colon. CA II and CA XII are highly expressed, whereas CA VII and IX normally show only weak staining (Niemela et al. Cancer Epidemiol Biomarkers Prev. 2007;16(9):1760-6. Kivela et al. World J Gastroenterol. 2005;11(17):2616-25. Kivela et al. Dig Dis Sci. 200;46(10):2179-86. Kivelä A et al. Am J Pathol. 2000;156(2):577-84.)

15. Page 10 line 230: cancers arising in the transverse colon were considered as left- or right-sided in the statistical analysis?
- We modified this section in the article to be more informative (page 12, lines 223-226): "Tumor location was also considered in the analysis: right side of the colon including the caecum, ascending colon and right flexure versus left side of the colon, including the left flexure, descending colon, sigmoid colon and rectum. Mid transverse tumors were excluded from the comparison."

16. It is strange that, despite CA IX is overexpressed in CRC (as shown in Figure 1), no correlation with survival was found. This detail needs to be discussed.
- We agree with the reviewer that this is indeed a strange phenomenon. Our original hypothesis was that CA IX could potentially show some correlation to survival, because of its higher expression in CRC and documented correlation results in several other tumor categories.
We added the following sentence (page 15, lines 287-288): "This finding highlights the unique biology of CRCs in terms of tumor development process."

17. Table1: please define "differentiation" (histological?).
- Table 1 has been modified to make this clear.

18. Finally, Authors should underline in the discussion that, although statistically significant, the value of HR is very close to 1, therefore CA expression may contribute only marginally to the prognosis of CRC.

- We have added the following sentence (Page 15, lines 284-285): "The obtained hazard rations were relatively modest, and thus the results need to be interpreted with caution." We have also modified the conclusion section according to this suggestion (Page 17, lines 344-346): "It is notable, however, that the hazard ratios were close to 1, and therefore CA expression may only marginally contribute to the prognosis of CRC."