

1. The referee 1 asks whether the same findings can be obtained in serrated carcinomas and serrated adenomas.

a. As we emphasized throughout the manuscript, our work is a preliminary study. Accordingly, we had to include a relatively limited number of cases. To make the data more reliable, we performed the study in patients with adenocarcinoma, not otherwise specified (NOS). Because this tumor is the most frequently diagnosed CRC worldwide. That is why, in order to ensure that the carcinoma group is composed of adenocarcinoma, NOS, we excluded not only other subtypes but also tumors carrying mucinous and serrated components focally. However, the fact that we designed our study in a single histological group does not exclude the requirement to receive the answer to this valuable scientific question asked by the referee through further research. Therefore, in the discussion, the importance of this question is emphasized as " Another essential factor to be considered is that this study was conducted only using samples from patients diagnosed with adenocarcinoma, NOS. Therefore, the relationship between Notch-1, Numb, Itch, and Siah-1 expression and their impacts on carcinogenesis and tumor behavior should be investigated in other histological subtypes of CRC as well as their precursors, particularly in subtypes with a more aggressive course, such as serrated carcinoma."

b. Depending on the revision described above in Materials and methods section (study design) the statement" The expression of Notch-1, Numb, Itch, and Siah-1 was investigated in 50 CRCs, 30 adenomas and 20 healthy colonic tissues diagnosed at the Department of Pathology." was corrected as " Expression of Notch-1, Numb, Itch, and Siah-1 was evaluated in 50 samples of CRC diagnosed as adenocarcinoma, not otherwise specified (NOS), in 30 adenomas (10 tubular, 10 tubulovillous and 10 villous), and in 20 healthy colonic tissues diagnosed at the Department of Pathology.."

2. The referee 1 criticized that the manuscript would be greatly enriched if the expression level of Notch1, Numb, Itch, and Siah-1 genes could be used to analyze the available data and compare them to the findings. According to this suggestion we handled the quantitative data about the relative gene expression levels and performed again our statistical analysis to compare them with available data. Therefore:

a. In the materials and methods section, the statement " Relative gene expression levels between the groups and their relationship with clinicopathological parameters were investigated by Student's t-test. " was added.

b. In the results section (2nd paragraph), after the first statement, the obtained results were presented as " The relationships between the gene expression levels of Notch-1, Numb, Itch, and Siah-1 and clinicopathological parameters are presented in Table 4, and the significant findings are illustrated in Figure 5. The level of Notch-1 gene expression was significantly higher in poorly differentiated and moderately differentiated tumors than in well-differentiated tumors ( $p<0.05$ ). This level was also 7-fold higher in tumors with lymph node metastases and 3-fold higher in advanced-stage tumors than in tumors without lymph node metastasis and in tumors with earlier stages, respectively ( $p<0.05$ ). Moreover, the mean level of Notch-1 gene expression was 8-fold higher in patients with shorter survival times than in patients who survived for more than 5 five years ( $p<0.05$ ). Overall, the expression levels of Numb, Itch, and Siah-1 were higher in well-differentiated tumors (6-fold increase), with lymph node metastasis (8-fold increase) and at advanced stages (5-fold increase) ( $p<0.05$ ). The survival of patients with higher Numb, Itch, and Siah-1 expression levels was better than that of patients with a low expression level of these genes ( $p<0.05$ ).

c. " Accordingly, the detailed information about this analysis is presented in a new table (Table 4). This revision and the suggestions of the second referee prompted us to revise Table 1 presented in the first submitted form of the manuscript. Therefore, new Table 1 includes the distribution of immunohistochemical findings among groups. The information about the gene expression levels (either numerical or dichotomized) in adenomas and carcinomas is presented in a new Table 3. A new figure 3 that presents the levels of gene expression of Notch-1, Numb, Itch, and Siah-1 in each case with CRC was also added. Figure 3 that is presented in the first submitted manuscript revised and numbered as Figure 4. Another new figure, Figure 5, is inserted to emphasize the significance of quantitative gene expression levels for four clinicopathological parameters (grade, lymph node metastasis, stage, and survival). Because of the revisions, Table 3, Figure 4, and Table 4 presented in the first submitted manuscript numbered as Table 5, Figure 6, and Table 6, respectively

3. Referee 2 asked for information about whether any difference exists in the anti-Notch-1 immunostaining between adenoma subtypes. In this study, we designed the adenoma group with an equal number ( 10 of each) of tubular, tubulovillous, and villous adenoma. We analyzed whether Notch-1, Numb, Itch, and Siah-1 expressions differ between these subtypes. However, we did not find any significant results. We considered this group as a preneoplastic lesion group due to the low number of cases and the study being a preliminary study. We preferred to

emphasize the other significant findings of the study. However, the following changes were made both in order to answer this question and in line with the existing comments:

a. In the materials and methods section (study design) the statement "The expression of Notch-1, Numb, Itch, and Siah-1 was investigated in 50 CRCs, 30 adenomas and 20 healthy colonic tissues diagnosed at the Department of Pathology." was corrected as "Expression of Notch-1, Numb, Itch, and Siah-1 was evaluated in 50 samples of CRC diagnosed as adenocarcinoma, not otherwise specified (NOS), in 30 adenomas (10 tubular, 10 tubulovillous and 10 villous), and in 20 healthy colonic tissues diagnosed at the Department of Pathology."

b. In the results section the statements " In terms of Notch-1, Numb, Itch, and Siah-1 expression, there was no significant difference between adenomas, healthy colon (controls), or tissues adjacent to carcinoma and adenomas ( $p > 0.05$ ) (Table 1). Similarly, the distribution of Notch-1, Numb, Itch, and Siah-1 expression among adenoma subtypes did not differ ( $p > 0.05$ ) (Table 1)." were added. Moreover, Table 1 is revised and the distribution of immunohistochemical staining of Notch-1, Numb, Itch, Siah-1 in subtypes of adenomas is presented.

4. It is pointed out to the importance of immunohistochemical expression of Notch-1, Numb, Itch, and Siah-1 in tissues adjacent to carcinoma and adenomas in the clarification of the role of these molecules in carcinogenesis. It is asked if there is any aberrant immunostained pattern in these regions. Similar to the distribution of immunohistochemical staining in subtypes of adenomas, any significant difference was not found between the staining of these adjacent areas and the distribution of staining in other groups. The data about the immunostaining of these tissues are given in the statements " In terms of Notch-1, Numb, Itch, and Siah-1 expression, there was no significant difference between adenomas, healthy colon (controls), or tissues adjacent to carcinoma and adenomas ( $p > 0.05$ ) (Table 1). Similarly, the distribution of Notch-1, Numb, Itch, and Siah-1 expression among adenoma subtypes did not differ ( $p > 0.05$ ) (Table 1). "in the results section. Additionally, Table1 is revised, and the distribution of immunohistochemical staining of Notch-1, Numb, Itch, Siah-1 in both tissues adjacent to adenomas and carcinomas is presented.

5. According to the comments of both referees English is reedited by AJE.