Dear Editor,

Please find enclosed our response to the reviewers’ comments.

Reviewer: Tamotsu Sugi

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached) and specific points:

1. The adenoma-carcinoma sequence model is generally accepted for polypoid tumors since it is common to find both adenomatous and carcinomatous lesions within such tumors. However, an increasing number of superficial of flat colorectal tumors have been reported, particularly in Japan. Recent molecular analysis indicates that there are two broad categories of colorectal tumors: superficial and conventional polypoid tumors. In contrast to polypid lesions, which follow the adenoma-carcinoma sequence, superficial colorectal tumors have distinct histological and genetic characteristics. The findings in this report suggest that colorectal adenomas are not homogeneous tumors. If possible, the author should examine expression levels of the three suggested genes not only in polypoid adenomas, but also in superficial adenomas.

Response:
This is a very good idea but unfortunately not possible in this pilot study. For a future larger study we will include superficial adenomas.

2. Colorectal carcinomas sometimes invade into the submucosa from the mucosa. If the author clarifies the point at which these two tumor grades (intramucosal cancer and submucosal cancer) of colorectal carcinoma acquire high expression levels of the genes examined, this manuscript could potentially contribute to the understanding of the underlying pathogenesis of colorectal tumors.

Response:
In this study we had 3 CRC tumors classified as Duke A and 2 classified as Duke B. For the remaining 4 CRC tumors it was not possible to obtain the classification. There was no significant difference in expression in lesions (or normal colonic mucosa) when we compared Dukes A and B tumors.
3. Colorectal tumors frequently contain considerable intervening stroma between neoplastic glands. The presence of nonneoplastic tissue also technically compromises mRNA expression analysis since the proportion of tumor cells in the specimen is reduced and DNA from nonneoplastic cells can complicate interpretation of mRNA expression data. Therefore, many of the problems limiting interpretation of mRNA expression analysis stem from contaminating nonneoplastic cells, which can lead to inaccurate analysis. The author should clarify the ratio of tumor cells to nonneoplastic cells.

Response:
Page 6, 6th sentence- The tumor histology of the adenomas was examined independently by two specialist histopathologists in order to determine the tumor stage as either mild/moderate (n=21) or severe (n=15) dysplasia (tumor cellularity 60-80%).

In this study mRNA was extracted from the most superficial tumor-rich areas, avoiding most of the deeper stroma-containing layers. Since we detected an increase in expression patterns between normal mucosa and tumor tissue we do not think that normal cell contamination is a major problem.

4. For the same reason, low expression levels of DNA repair genes in the normal mucosa may be influenced not only by normal crypt cells, but also by low levels expression of interstitial cells. The author should discuss expression of the three genes in normal crypt cells.

Response:
A study by Hengstler et al, 1998 detected a relatively large variation in gene expression when they used isolated mucosa (dissected off the submucosa) from different sites of the same colonic tissue, whereas the variability of total colon tissue taken from different sites was much smaller. In order to obtain a better reproducibility they examined total colon tissue, which should not be interpreted as cells of origin for the corresponding tumor, but which represents a homologous control tissue which gives information on constitutive expression of the examined factors.

In our study we used normal colonic mucosa and have used this as a homologous control tissue.

We have replaced the phrase “normal tissue” with normal colonic mucosa throughout the text.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct):

1. The introduction is rather long and should be shortened
Response: Page 4, sentences 3-6 is taken out of the introduction.

2. There are a few missed characters in this manuscript

Response: The manuscript has been proofread before resubmission.

Reviewer: Sima Salahshor

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached) and specific points:

1. DNA repair mechanisms are complex and involve more than 100 genes. In the background section it is not clear why authors choose to study specifically these two DNA repair genes.

Response: Page 4, 6th sentence- DNA repair mechanisms are complex and involve several genes. OGG1 and ERCC1 are among the few for which it has been shown that mRNA levels and repair activity correlate.

Page 4, 9th sentence- The mRNA levels of ERCC1 may correlate with DNA repair capacity in various tissues (Dabholkar, Bostick-Bruton et al. 1993; Cheng, Guan et al. 1999; Li, Yu et al. 2000; Vogel, Dybdahl et al. 2000), and also predict the response to chemotherapy (high ERCC1 levels have been associated with tumor growth and lower levels have been associated with improved disease response) (Metzger, Leichman et al. 1998; Li, Yu et al. 2000; Shirota, Stoechlmacher et al. 2001; Lord, Brabender et al. 2002; Rosell, Taron et al. 2004)

Page 5, 6th sentence- Previous studies have shown that oxidative stress is generally proportional to OGG1 mRNA expression and activity (Tsurudome, Hirano et al. 1999; Kim, Morimoto et al. 2001; Bancroft, Lupton et al. 2003).

2. A number of studies have examined the levels of these three proteins in different forms of cancer, including colon cancer which should be cited in this paper


3. OGG1 gene is coding for over 8 different splice variants. Are the primers used for quantitative RT-PCR detects all the variants of some of them? (Genebank Accession number?)

Response:
Page 7, 2nd sentence- The OGG1 probe recognizes all splice forms of OGG1.

4. As mentioned in the text there is 5-10 fold variation in mRNA ERCC1 and OGG1 levels measured in healthy volunteers (page 11-Ref. 23). If you measure mRNA levels in tumors/adenomas compared to the corresponding normal tissue (for each case), in how many cases do you find statistically significant difference?

Response:
The 5-10 fold variation measured in healthy volunteers (page 11-Ref. 23) gives a description of variation between individuals. The measured mRNA levels in lesions compared to corresponding normal colonic mucosa gives a description of expression within cases were we detected an increase for all lesions. In addition to the p values (Table 2) we have included the confidence intervals for all three genes in order to present the distribution of the difference between normal colonic mucosa and lesions.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. The page number is missing in some of the references. Reference number 33 is published in 2006 and not 2005.

Response:
Page 17, 2nd sentence- Reference 33 has page number 229 (1):85-91.
2. Please provide the full names of OGG1 and ERCC1 in the beginning of the paper.

Response:
Page 4, 6th sentence- Previous studies have indicated that the NER gene excision repair cross-complementation group1 (ERCC1) and BER gene 8-oxoguanine glycosylase 1 (OGG1) may be inducible.

Discretionary Revisions

1) It has been suggested that the mRNA and protein levels of DNA repair genes correlate. Immunohistochemistry or western blot analysis of OGG1, ERCC1 and RAI in tumors and corresponding normal tissues would provide evidence whether increased mRNA level in tumors is also an indicator of higher levels of protein.

Response:
Good idea, but unfortunately not possible at the moment.

2) Authors suggest that the low level of DNA repair genes in normal tissue is a risk factor for adenoma formation. Have they compared the expression level of these genes in normal tissue in general population (control) with their levels in normal tissue from cancer patients?

Response:
We did try, but low amounts of available RNA from healthy volunteers made it impossible for us to determine the levels of these genes, since they are expressed at very low levels.

3) Are there any clinical data available to assess whether the high level of DNA repair genes in the cases studied there is a predictor of survival or respond to therapy?

Response:
Data are not available.

4) Have authors any explanation why cases with low expression of these DNA repair genes in normal tissue show increase expression in tumors? It is possible that these results indicate that ERCC1, OGG1 and RAI are functioning normal and are activated in tumors as a result of the disease?

Response:
We interpret the data as follows; the increased expressions of ERCC1, OGG1 and RAI indicate that already in the mild adenomas, cellular stress is greatly increased. Since there is no trend in the expression pattern from mild adenoma over severe adenoma to carcinoma, there is no indication that the stress is worsened. On the other hand, we observe a tendency towards less expression in normal tissue. This can be interpreted in two ways; either that the low expression was pre-existing and thus may be a cause of the disease or that it is a consequence of the disease. In the latter case, we have no explanation for the observation.

Regards,
Elin H. Kure