Author's response to reviews

Title: Differential Expression of Colon Cancer Associated Transcript-1 (CCAT1) Along the Colonic Adenoma-Carcinoma Sequence

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Author's response to reviews:

Dear Sir,

Attached, please find our revised manuscript entitled:

"Differential Expression of Colon Cancer Associated Transcript-1 (CCAT1) Along the Colonic Adenoma-Carcinoma Sequence"

We thank the reviewers for an excellent review.

We have revise the manuscript accordingly, with the following responses:

Reviewer #1:

Comments:

1. "CCAT1 is a non-coding RNA and a potential specific biomarker for colorectal cancer. The present study was to define and validate CCAT1 as a CC-specific biomarker, and to study CCAT1 expression in various stages during the CC tumorigenesis. The results showed that the expression of CCAT1 was higher in adenomatous polyps than in CC tissues, and the most highly CCAT1 expression was found in metastases tissues such as LN and peritoneal metastases. Liver metastases tissues also showed a higher expression than CC. The results indicate that CCAT1 expression is more significant for adenomatous polyps or metastases than CC. Then could it be an useful marker for CC?"
Response: We believe that based on the data presented in this manuscript, showing CCAT1 to be uniformly unregulated in colorectal cancer tumor tissues as well as in various disease stages, it may be used as a biomarker.

Since, the current study focuses on expression in tissues, we believe that CCAT1 can be used to differentiate difficult to diagnose lesions such as metastasis of unknown origin.

If we will be able to trace CCAT1 in blood samples of patients with adenomatous polyps and early cancers, this may be used as a biomarker for diagnosis. However, this is out of the scope of the current work. The results presented herein provide the basis for future studies that will establish the role of CCAT1 as a biomarker for CRC.

Comment: "But the results were interesting. I suggest the authors further investigate the following questions:

1) If the patients with high CCAT1 expression were more easily to develop into CC?"

Response: This is a very interesting question, but since we studied adenomatous polyps that were removed, there is no way we could learn what have happened if they were left in place. Obviously, conducting a study where adenomatous polyps are left intact and not removed is unethical and therefore impossible to conduct.

"2) The authors should study the expression of CCAT1 in tissues of CC and the matched metastasis nodes(LN, liver or peritoneal ) in the same patients. Generally, they should have a consistent expression style."

Response: We fully agree with the reviewer. However, as much as we tried, during the study period to collect tumor, lymph nodes, and distant metastasis from the same patient, we were not been able to obtain sufficient amount of such tissue samples. All adjacent normal tissues were obtained from the same patients with the primary tumor tissues. We are currently trying to collaborate with other medical centers to obtain such tissue sets but those are extremely hard to find. In the current study, we have three patients with matched tumor tissue and lymph node metastasis. All three had upregulated CCAT1. The following paragraph was added to the text:

"In three patients (patients #612, #655, and #698) we had matched tissues of primary tumor and lymph node metastasis. CCAT1 expression was up-regulated in all three primary tumors (Table 3)."

Comment: "Since its role of CCAT1 is not well known now, if the expression
CCAT1 was associated with metastasis deserved to be investigated."

**Response:** Since CCAT1 is upregulated in 90% of the primary tumors studied, a simple "expressed or not expressed" analysis is not going to be of benefit in defining the metastatic potential of a given tumor based on CCAT1 expression. We are currently studying a large cohort of patients (n=100) in order to try and quantitate the level of CCAT1 expression and correlate it with different clinical parameters such as presence of metastasis, disease recurrence and survival. However this prospective trial has just started and we will have this important data after the trial will be completed.

Comment: "2. In the part of materials and methods, there are 113 tissue samples for last analysis. But in the abstract and results, the sum of the tissues seemed to be 103. Please have a check of it."

**Response:** Thank you for your comment, the additional 10 samples were the normal lymph nodes (n=10) obtained from the same CRC patients with lymph node metastasis. Sorry if this was not clear; in the abstract we have modified the number of lymph nodes. In the body of the paper and in Figure 3 it is stated that there were 10 normal and 10 metastatic LNs. Again, sorry for the misunderstanding.

Comment "3. The writing of the manuscript should be more smoothly. Several mistakes were found, such as “adnom(a) adenoma), heaptic(hepatic), patholgist(pathologist),tumer(tumor)......”.

**Response:** Thank you, we are sorry for the typos, all were correct and the manuscript was reviewed by an English speaking editor.

Comment: "4. As for the statistical analysis methods, please have a confirmation that if the data accord with logarithm normal distribution. The scattering of the data is a little wide. I suggest the authors have a consultation of the specialist of statistics."

**Response:** Thank you, we have consulted with Dr. Robin Howard, a biostatistician.

Reviewer #2:

Comment:

"Major revision:

In their manuscript entitled “Differential Expression of Colon Cancer Associated Transcript-1 (CCAT1) Along the Colonic Adenoma-Carcinoma Sequence”, the authors detected CCAT1 expression across the adenoma-carcinoma sequence of CC tumorigenesis, and found that CCAT1 is up-regulated in the colon
adenoma-carcinoma sequence, through all disease stages, including advanced metastatic disease. The authors concluded that CCAT1 may serve as a biomarker for diagnosis, staging and follow-up of CC.

Comment:
"Major problems:
1. The language should be polished."

Response:
Thank you, the manuscript was edited by an English speaking editor.

Comment:
"2. Some abbreviations are abused. For example, CCAT1 and qPCR, and so on."

Response:
As the title of the article provides an open as well as an abbreviated version of Colon Cancer Associated Transcript-1, we did not feel the abbreviation would be inappropriate throughout the manuscript. qPCR has been modified to include the abbreviation for reverse transcription (RT), and is thus now, qRT-PCR. Both abbreviations are listed together with others used in the paper.

"3. The method used in this article is correct; however, if CCAT1 is up-regulated in the colon adenoma-carcinoma sequence, through all disease stages, including advanced metastatic disease, maybe it is not a good biomarker for diagnosis, so the conclusion should be rewritten."

Response:
The aim of this work was to study in what stages of disease CCAT1 is expressed, and to what level. The fact that CCAT1 is expressed in all stages of disease form pre-malignant to metastatic disease does not exclude the possibility that it can be a good biomarker. On the contrary, if we can show in future studies that CCAT1 can be detected in sera of all patients with CRC, it can be used for diagnosis. This might not contribute to early detection, but in case of a positive result, the patient will be sent to colonoscopy followed by cross-sectional imaging for tumor localization and staging. CCAT1 testing may also be used for follow up, to monitor recurrence. However, we agree with the reviewer that testing these is beyond the scope of this manuscript and we changed our conclusions accordingly.

The new conclusions are;
"CCAT1 is up-regulated in the colon adenoma-carcinoma sequence. This
up-regulation is evident in pre-malignant conditions and through all disease stages, including advanced metastatic disease suggesting a role in tumorigenesis and the metastatic process."

Comment:

"4. The Introduction should be rewritten. For example, the paragraph “The most common application of CEA...” is useless. The paragraph “Adjuvant treatment selection for patients…” is also suitable. The focus should be introduce the advance of CRC biomarkers"

Response:

The introduction was changed according to the reviewer's suggestion.

The following paragraph was added to the text.

" There is an increasing number of microRNA fragments found in CC primary tumor tissues, metastasis, and plasma [14-17] that may serve as biomarkers for the detection of CC, estimating prognosis, and use in the follow up of CC patients to assess treatment response and disease state [18]."

Comment:

"5. The authors should compare diagnostic efficacy using CCAT1 with CEA and/or CA199."

Response: The discussion was modified according to the reviewer's suggestion.

"Serum markers in clinical use for CC (CEA and CA 19-9) are neither sensitive nor specific [40]. Therefore the most common application of CEA and CA-19-9 is to monitor patients for recurrent disease following treatment of CC or to monitor response to systemic therapy [41]. If the measurement of CCAT1 levels in the plasma of CC patients should prove both feasible and reproductive, then it may be added to the current serum markers to monitor disease behavior and patient response to treatment. "

Sincerely yours,

Prof. Aviram Nissan