

**Title: Connective Tissue Growth Factor Expression Hints at Aggressive Nature of Colorectal Cancer**

**No. 70199**

Dear editors,

We are very thankful for your time and thoughtfulness in reviewing our paper. We appreciate your response and overall positive initial feedback and made modifications to improve the manuscript. After carefully reviewing the comments made by the Reviewers, we have modified the manuscript to improve it. We hope that you will find the revised paper suitable for publication, and we look forward to contributing to your journal. Please do not hesitate to contact us if you have any further questions or concerns regarding the manuscript.

Thank you for your careful consideration of our manuscript.

Best Regards

Yours Sincerely

Dr. Syed Mudassar  
Professor and Head  
Department of Clinical Biochemistry, SKIMS  
Email: [syed.mudassar@skims.ac.in](mailto:syed.mudassar@skims.ac.in)

## **Peer-review report**

Reviewer #1:

**Scientific Quality:** Grade B (Very good)

**Language Quality:** Grade A (Priority publishing)

**Conclusion:** Minor revision

### **SPECIFIC COMMENTS TO AUTHORS**

Comments on the manuscript: "Connective Tissue Growth Factor Expression Hints at Aggressive Nature of Colorectal Cancer" Connective tissue growth factor (CTGF) works in many cellular mechanisms. In order to understand its role in colorectal cancers (CRC), the authors studied its expression and its localization in tumor cells. They also associated these results with several clinicopathological parameters. For this, the authors used 71 tissue samples from histopathologically confirmed CRCs as well as adjacent normal tissues serving as controls. The samples were analyzed with several methods: real-time PCR, immunohistochemistry and Western blot. The results were statistically analyzed in relation to the clinicopathological data of the patients. In conclusion, the study showed that CTGF was overexpressed in colorectal tumors. Its expression could be an independent prognostic factor for patients with CRC. this article is useful and interesting. The conclusion is cautious. It may be published after however some improvements. Here are a few remarks. Page 3, introduction: What is the difference between transitioned and transitional country? Page 3: in the sentence "The acronym CCN was assigned to this family after the initials of ist three proteins", what is "ist" ? Is it "its"? Page4, patient specimens: give here, how many males and females were studied. (See results). Page 5: Protein extraction and Western Blot Assay : for centrifuge speed, use number of g instead rpm. Page 6: specify the method for deparaffinising the sections. Page 10: " This was also the ist study which revealed that the presence of lymphovascular invasion", what is "ist" ? Is it "first"? Pages 15-16, table 2. Check the legend and verify if all the elements of the table are given in the legend and if all the elements of the legend are in the table. I didn't find "Or", "\*= Statistical Significance=P<0.05" in the table; and I didn't find R and L in the legend. Pages 17-18, table 3. idem : Check the legend and verify if all the items of the table are given in the legend and if the items of the

legend are in the table. Pages 18-19, table 4. idem : Check the legend and verify if all the items of the table are given in the legend and if the items of the legend are in the table. Pages 20-21: explain "CTGF" in the legend. Page 22: Add a scale bar on each picture. References. Check references and verify if they are presented according to the journal standard.

### **RESPOND TO REVIEWER**

Page 3, introduction: What is the difference between transitioned and transitional country?

REPLY: Transitioned and Transitional Country means developed and developing countries.

Page 3: in the sentence "The acronym CCN was assigned to this family after the initials of ist three proteins", what is "ist"? Is it "its"?

REPLY: Yes, ist means "First"

Page 4, patient specimens: give here, how many males and females were studied. (See results).

REPLY: 38 males and 33 females

Page 5: Protein extraction and Western Blot Assay: for centrifuge speed, use number of g instead rpm.

REPLY: Replaced rpm with g in manuscript as  $7000\text{rpm} = 3567\text{g}$ ,  $1000 = 73\text{g}$ , (Radius of our centrifuge = 65mm)

Page 6: specify the method for deparaffinising the sections

REPLY: DEPARAFFINIZATION: The slides must be deparaffinized and rehydrated before beginning the staining procedure. Paraffin removal that is not thorough might result in poor staining of the section. We used Xylene and Ethanol for deparaffinising the sections as follows;

Placed the slides in a rack, and performed the following washes:

1. Xylene: 2 x 3 minutes
2. Xylene 1:1 with 100% ethanol: 3 minutes
3. 100% ethanol: 2 x 3 minutes
4. 95% ethanol: 3 minutes
5. 70 % ethanol: 3 minutes
6. 50 % ethanol: 3 minutes
7. Running cold tap water to rinse
8. Kept the slides in the tap water until ready to perform antigen retrieval

Page 10: “This was also the 1st study which revealed that the presence of lymphovascular invasion”, what is “1st”? Is it “first”?

REPLY: Yes 1st means First

Pages 15-16, table 2. Check the legend and verify if all the elements of the table are given in the legend and if all the elements of the legend are in the table. I didn't find “Or”, “\*= Statistical Significance= $P < 0.05$ ” in the table; and I didn't find R and L in the legend.

REPLY: 1. Please find the parameter, “Source of drinking water” where R= River water through Tap and L=lake water through Tap.

2. \*=Statistical significance, actually we had kept \* mark for significant values firstly, but then we changed them and kept in Bold, but forgotten to write it in the legends.

3. O.R is for Odds ratio, but as we have already written Odds ratio in full text form, so we deleted O.R legend.

Pages 17-18, table 3. idem : Check the legend and verify if all the items of the table are given in the legend and if the items of the legend are in the table.

REPLY: All checked and modified accordingly.

Pages 18-19, table 4. idem : Check the legend and verify if all the items of the table are given in the legend and if the items of the legend are in the table.

REPLY: All checked and modified accordingly.

Pages 20-21: explain “CTGF” in the legend.

REPLY: Explained “CTGF” as Connective Tissue Growth Factor

Page 22: Add a scale bar on each picture. References. Check references and verify if they are presented according to the journal standard.

REPLY: We have taken the images on high power (40X) and low power (10X) and a scale bar of 100 $\mu$ M. (Added in image)

References;

According to Journal Standard

*#Science editor:*

The authors of this basic study claimed that the expression of CTGF was increased in CRC and was linked with poor overall and disease-free survival of CRC patients. It will be helpful if one more reviewer can be found. The quality of Figures 3 and 4 are poor, which can be improved after revision. In addition, the green background in Figure 7 is not necessary. Please ask the authors to revise according to the reviewer's comments.

Language Quality: Grade B (Minor language polishing)

Scientific Quality: Grade B (Very good)

We have replaced the Figure 3 and 4 with high quality figures, and removed the green background from Figure 7. The manuscript was edited by a native English speaker for language spelling, grammar, and punctuation.