Title: The high affinity selectin glycan ligand C2-O-sLex and mRNA transcripts of the core 2 beta-1,6-N-acetylgalactosaminyltransferase (C2GnT1) gene are highly expressed in human colorectal adenocarcinomas

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Reviewer: Maria V Croce

Reviewer's report:

This paper is a welcome addition to Oncology and its originality is based on the study of the C2-O-Lex that can be considered as a tumor marker in human colorectal adenocarcinoma.

Major Compulsory Revisions:

1- The authors present confused arguments in relation to increased CHO-131 mAb reactivity.

In the Abstract, they state: “Positive reactivity with CHO-131 mAb was very prominent in neoplastic colorectal glands of well to moderately differentiated adenocarcinomas.” (page 3, paragraph 1, lines 1-3) which agrees with Results, page 13, paragraph 1, lines 5-10: “As colorectal progressed from well to poorly differentiated, proportionately greater numbers of carcinomas displayed areas of solid tumor growth that lacked glandular structures and stained less intensely with CHO-131. Thus, the positive reactivity with CHO-131 mAb was most prominent in neoplastic colorectal glands of well to moderately differentiated adenocarcinomas (Fig.2-3 and Table 1).”

The combination of moderately and poorly differentiated tumors for the statistical analysis does not appear adequate because it may have masked the results as they have clearly shown above. In this sense, the results should be presented separately, Fig. 5A should be changed as well as paragraph 3 on page 14, lines 17-22: “The combined group…”

Regarding the paragraph included on page 14, paragraph 3, lines 15-17: “In 31 of 66 of these moderately and poorly differentiated carcinomas with high CHO-131 reactivity, greater than 50% of the total tumor mass in each tissue positively reacted with CHO-131”, should it be understood that in poorly
differentiated colonic adenocarcinomas, although solid tumor areas are greater than in moderately ones, they displayed positive staining in more than 50% of the total tumor area?

2- The organization of the article should suffer some changes.

Methods:
Before the item Antibodies a subtitle such as Tissue samples should be inserted. All data about samples used for both immunohistochemistry (IHC) and for RT-PCR should be included there.

After that, the item Immunohistochemistry and Histology should be separated, in first place Histopathology and then the Immunohistochemistry.

The sentence: “Tissue sections…paraffin” on page 7, paragraph 2, lines 13-23, should be included in Histopathology as well as the paragraph 2, on page 11, lines 4-9, including the numbers of well, moderately and poorly differentiated tumors employed for IHC and for RT-PCR separately.

Besides, the sentence: “To evaluate…procedures” on page 8, paragraph 1, lines 4-6, should be included in Histopathology.

The subtitle Immunohistochemistry should begin with the procedures employed for all the antibodies: deparaffination, etc; only then go on with the description of C2-O-sLe detection: “To detect…15 ug/ml.” (page 7, paragraph 2, lines 23-24) followed by paragraph 2, lines 10-23, on page 8, and then the positive control for C2-O-Lex, paragraph 2, lines 19-25, page 9.

After that the description of CEA and Lu-5 procedures beginning with the sentence: “As a positive …0.5ug/ml” on page 7, last line and on page 8, paragraph 1, lines 1-4 should be included. The sentence: “Tissue sections…above” on page 8, paragraph 3, line 25 and on page 9, paragraph 1, line 1 should be removed.

Results
The paragraph: “We wanted to…(n=8)” should be removed.

Statistical review: It must be adequate to the above considerations.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Declaration of competing interests:
I declare that I have no competing interests.