Author's response to reviews

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

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Version: 3 Date: 23 January 2009

Author's response to reviews: see over
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Version: 2 Date: 23 January 2009

Author’s Response to Reviews: see over
Reviewer's report

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

Version: 1 Date: 15 September 2008

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…”

- A new graph was created (and is labeled Fig. 6A) with the well, moderately, and poorly differentiated groups of colorectal adenocarcinomas separated. Statistical analysis (Pearson’s chi-square test) was performed on the data. We found that moderately differentiated carcinomas with high positive CHO-131 mAb reactivity displayed significantly higher levels of staining with CHO-131 mAb than well differentiated adenocarcinomas with high positive CHO-131 mAb reactivity using the Pearson’s chi-squared test (p-value = 0.04, odds ratio = 0.38). A difference was not found in CHO-131 mAb staining between moderately and poorly differentiated adenocarcinomas and this was attributed to the small sample size (n = 8) for the latter group. The text was revised appropriately (page 14, line 12).

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?

- Our statements about CHO-131 reactivity and tumor progression have been modified for clarity, (page 13, line 17). We assessed the reactivity of CHO-131 mAb with 8 poorly differentiated colorectal adenocarcinomas. Six of 8 adenocarcinomas were highly reactive with CHO-131 mAb at a 2+ to 3+ staining intensity. Two of these 6 tissues displayed 3+ staining intensity with CHO-131 mAb where 95-100% of the total tumor area consisted of positively stained glandular structures. The remaining 4 tissues displayed extensive areas of solid tumor mass where only 5-20 % of the total tumor area contained glandular structures that reacted positively with CHO-131 mAb. In two of 8 poorly differentiated colorectal adenocarcinomas we observed low (1+) reactivity with CHO-131 mAb and these tissues had minimal glandular structures (less than 10 % of the total tumor mass).

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.

- This section was modified to begin with the subtitle ‘Tissue Samples’ and further categorized into ‘Histology’ and ‘Immunohistochemistry’ sections before the subtitle ‘Antibodies’. Data about samples used for these procedures were included in the appropriate sections.

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 sentences were revised and placed in the appropriate sections as suggested (page 8).

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.

- The Immunohistochemistry section was revised as suggested (page 9, line 4).

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

- In the Results section the paragraph ““We wanted to…(n=8)” was removed as suggested.

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

- The statistician Qing Cao, a member of the Biostatistics Core, Masonic Cancer Center at the University of Minnesota was consulted and all aspects of statistical review for the manuscript were reanalyzed. She is included as a co-author on the paper. The paragraph on the statistical methods titled “Statistical Analysis” was modified (page 12, line 10).

**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.
**Reviewer's report**

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

**Version:** 1  **Date:** 17 September 2008

**Reviewer:** Fabio Dall'Olio

**Reviewer's report:**

Major compulsory revisions

A comparison of the reactivity between CHO-131 and a "usual" anti sLex antibody, such as csLex1, on some selected specimens is necessary to understand the differences in the pattern of expression of "general sLex" and C2-O-sLex epitope. For example, 1 sample for each of the three differentation classes (well, moderately and poorly) and for each class of staining reactivity (from 0 to 3), total 12 samples. The same for metastasis (other 12 samples). This is very important to establish whether the reactivity of the two antibodies are overlapping or not.

- We stained 134 colorectal adenocarcinomas, 38 metastatic liver tumors, 5 sections of normal colonic mucosa, and 5 sections of normal liver with CSLEX1 mAb (described in Methods page 8, line 12). We found that the reactivity of CSLEX1 mAb overlapped with that of CHO-131 mAb. Positive staining with CSLEX1 mAb was more widely distributed than that for CHO-131 mAb in malignant tissues. This result was expected because the sLe\[^x\] epitope can be present on macromolecules such as glycolipids and other glycoproteins in addition to O-glycans. In contrast to the staining pattern seen with CHO-131 mAb, the reactivity of CSLEX1 mAb was not correlated with differentiation status of the tumor (page 15, line 21) indicating that positive reactivity CHO-131 mAb was a more specific indicator of an advanced tumor.

Fig. 5 is confusing and should be deleted. The piece of information they report is already given by Table 1.

- Figure 5 was modified to address the suggestions of Dr. Dall'Olio and those of the other reviewer. This figure is labeled Fig. 6 in the revised manuscript. The results are described on page 14, line 12 and on page 15, line 23.

Minor essential revisions

The level of expression of C2-O-sLex is higher in less differentiated cancers. On the other hand, the level of C2GnT1 mRNA seems to be lower in less differentiated cases. This apparent discrepancy could be due to the fact that different groups of patients have been analyzed but also to the fact that in the less differentiated cases the percentage of tissue with glandular structures is
decreased. This point should be briefly discussed.

- This discussion is included in the manuscript on page 18, line 1.

The mucin type Core 2 GnT (C2GnT2) has been found to be downregulated in colon cancers (Huang et al, Oncogene 25 3267-3276, 2006). Since the two enzymes can be involved in the biosynthesis of the same structure, their opposite regulation in colon cancer tissues should at least be discussed.

- This discussion is included in the manuscript on page 19, line 15.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

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