Title: Expression of the Na+/I- symporter (NIS) is markedly decreased or absent in gastric cancer and intestinal metaplastic mucosa of Barrett's esophagus

Authors:

Aron Altorjay (altarjay@mail.fmkorhaz.hu)
Dohan Orsolya (odohan@gmail.com)
Anna Szilagyi (szilagyi@mail.fmkorhaz.hu)
Monika Paroder (mparoder@aecom.yu.edu)
Irene L Wapnir (wapnir@stanford.edu)
Nancy Carrasco (carrasco@aecom.yu.edu)

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Dr. Iratxe Puebla
Senior Assistant Editor
BMC-series journals
BioMed Central Editorial

Re: manuscript 6641533551154289

Dear Dr. Puebla:

Thank you for your recent message with the reviewers’ comments on our manuscript originally entitled “Expression of the Na⁺/I⁻ symporter (NIS) is markedly decreased or absent in gastric cancer and precancerous lesions.” We were pleased to see the reviewers’ prevailing favorable evaluations of our research. Below are our point-by-point responses to all the reviewers’ comments and detailed descriptions of the revisions we have made in the manuscript.

Reviewer 1, Dr. Toshiyuki Nakayama:

Major Compulsory Revisions

1. We agree with Dr. Nakayama’s suggestion to change the title of our manuscript to “Expression of the Na⁺/I⁻ symporter (NIS) is markedly decreased or absent in gastric cancer and intestinal metaplastic mucosa of Barrett’s esophagus” and have therefore made the change. The new title informs the reader what specific precancerous lesion is involved.

2. As requested by Dr. Nakayama, we are now indicating the number of each histological type of gastric cancer examined. On page 9, it now says: [adenocarcinoma (n=4), signet-ring cell (n=3), papillary (n=1)]. We also agree with Dr. Nakayama's comment regarding the diagnosis of gastric squamous carcinoma; the correct diagnosis is gastric adenocarcinoma. We have made the correction throughout the manuscript, i.e., on page 9, paragraph 2, line 2 and page 18, paragraph 2, lines 9-11, it now says gastric adenocarcinoma.

3 and 4. Dr. Nakayama is no doubt correct in pointing out that it would be of considerable interest to study NIS expression in gastric adenomas, which are indeed precancerous lesions, and in intestinal metaplastic mucosa in the stomach. However, such studies are not necessary for our conclusions to be valid or for our observations to be relevant to gastric cancer. Our aim was to analyze NIS expression in a wide variety of gastrointestinal tract pathologies, not in all of them. Our view is that the studies suggested by the reviewer should be undertaken in the near future.
5. As recommended by the reviewer, we are now indicating that all the gastric polyps we investigated belong to the hyperplastic category (see page 9, last paragraph, line 1 and Table 2 under "stomach").

6. We do not have any data on gastric polyps with colon-type metaplasia. As indicated above (#5), all the gastric polyps we investigated were hyperplastic.

**Minor Essential Revisions**

1. Dr. Nakayama is right. We had made a typographical error. As he suggested, we have corrected the number of samples that exhibited faint focal expression >3 cm away from the gastric tumor from 3 to 2 in Table 3.

2. Fig. 1F was not deformed. It was only minimally fitted to the panel frame. No change is necessary.

**Typographical**

1. We deliberately set “secretion” in Italics, for there are still publications incorrectly stating that NIS traps I- from the gastric lumen.

2. The parenthesis has been inserted (page 10, line 21).

3. "negativ" has been corrected to "negative" (page 18, line 10)

4. "intestina" has been corrected to "intestinal" (page 20, line 2 in Table 2).

**Reviewer 2, Sebastiano Filetti**

**Minor Essential Revisions**

1. We agree with Dr. Filetti’s comment, which is why we cautiously stated even in the original version of the manuscript (abstract, lane 4 from bottom) that "NIS may prove to be a significant tumor marker in the diagnosis and prognosis..." In all the tumor samples investigated by immunoblot, NIS expression was either absent or significantly weaker than in normal tissues. Clearly, in the tissue samples used for immunoblot analysis, it was not possible for us to entirely separate tumoral tissue from the surrounding transitional and normal tissues. This explains the weak positivity of 5 out of 17 samples. If only tumoral tissue had been present, we would have most probably observed even less NIS expression. This is in agreement with our immunohistochemistry findings, i.e., that NIS positivity was detected only at the border of the tumors, never in the tumors themselves. The study of Wapnir et al was performed on tissue microarrays, containing small tissue cores. While the microarrays are excellent tools for screening large numbers of samples, the small size of the tissue cores does not allow for comparison of normal, “border," and tumoral tissues from the same specimen. Indeed, this was one of the reasons we decided to expand our studies in the GI system using conventional tissue sections.

2. As suggested by Dr. Filetti (Introduction, page 3, line 11 and Discussion, page 9, line 4), we have added the placenta as one of the tissues that transport I- and express NIS, including the suggested reference by Bidart et al: [JM Bidart, L Lacroix, D Evain-Brion, B Caillou, V Lazar,

3. We understand the reviewer’s point and we have included the reference from Bruno *et al* on page 3, at the end of paragraph 1. Yes, it is well known that radioiodide is accumulated in the stomach. However, we initially included and would still prefer to keep Fig. 1A because, surprisingly, there are published reports incorrectly asserting that gastric NIS “traps I− from the gastric lumen” [see, for example, Kotani *et al* (Immunol Immunopathol, 1998 89:271-8]. In our manuscript, it is important that the readers are aware that, in gastric mucosa, I− is taken up via NIS from the bloodstream and then secreted to the gastric lumen.

4. As per Dr. Filetti’s suggestion, we mention the findings by Wu *et al* on page 4, line 6 from the bottom.

5. No, the samples examined by immunoblot did not come from the same patients as those analyzed by immunohistochemistry. This point is now made clear on page 8, last line.

Discretionary revision

6. Although the hypothesis of Venturi *et al* is interesting, we have decided not to include a discussion of it in our manuscript.

7. Even though we understand Dr. Filetti’s point, we have opted to keep paragraph 2 on page 11 because it provides helpful information for prospective readers who have no extensive endocrinology background.

**Reviewer 3, Christine Spitzweg**

*Minor Essential Revisions*

1. As suggested by Dr. Spitzweg, we now refer to Fig. 2 on page 8, paragraph 1, line 7 and paragraph 2, last line.

We trust that you and the reviewers will find our responses and revisions to be satisfactory. We thank the reviewers for their helpful comments.

We look forward to hearing from you. Thank you for all your assistance.

Best regards,

Nancy Carrasco, MD
Professor