Author's response to reviews

**Title:** N-Acetylgalactosaminyltransferase-14 as a potential Biomarker for Breast Cancer by Immunohistochemistry

**Authors:**

Chen Wu (dawnwuchen@163.com)
Xiaodan Guo (guoxiaodan1985@yahoo.com.cn)
Weina Wang (wnlj1973@sina.com)
Yun Wang (Yunwang75@sina.com)
Zhongcheng Liu (liuzc@hbu.edu.cn)
Bo Zhang (bob040306@163.com)
Wenqian Song (songwenqian2008@sina.com)
Jianfeng Ge (869379139@qq.com)
Hao Deng (DDhao1988@163.com)
SLSi Ma (sisimama123@163.com)

**Version:** 4  **Date:** 6 February 2010

**Author's response to reviews:** see over
Feb 06, 2010

Biomed Central Editor

Dear Editor,

We sincerely appreciate the reviewer’s comments. The concerns of the reviewers and their suggestions for improvement of the manuscript have been addressed. According to the comments, we do additional experiments. The manuscript was revised. Below we provide a point-by-point response to the comments.

We are looking forward to your reply.

Sincerely

Chen Wu, Ph.D
Hebei University
Baoding

E-mail: dawnwuchen@163.com
Referee I

Major points:

a) Since this is a recently discovered enzyme, the antibody should be tested thoroughly in advance. I suggest to perform a Western blot analysis to show the specificity of the antibody. Ideally, only one band should be visible.

Response

A western blot had been performed in our previous work. The result showed that the antibody was specific. In this experiment, we found that GalNAc-T14 was expressed endogenously in human kidney carcinoma cell line 786-O cells.

Figure 1. The expression of GalNAc-T14 protein in 786-O cells transfected by pCI-GalNAc-T14 or pCI-neo determined by western blot (GAPDH as an internal control)

1. lysis of 786-O cells transfected with pCI-GalNAc-T14
2. lysis of 786-O cells transfected with negative control pCI-neo
b) The sample size is pretty low. I strongly suggest to increase the sample size at least to N=30 of each the cancer cases and normal controls.

Response

Thank you for your comment. We acknowledge the limitations of the sample size. So we did our best to increase the sample size in a limited time for revision (about one month). As a result, 20 invasive ductal carcinomas, 14 adenosis and 2 mucinous adenocarcinoma were collected. In the revised manuscript, we increased the sample size of the cancer case (invasive ductal carcinomas, N>30) and the benign control (adenosis, N>30). We are sorry that the samples of the other cancer cases can not be collected. (It is easier to collect the cancer cases of invasive ductal carcinomas and adenosis relative to other cancer cases and normal breast tissue). Even so, we can see the difference of GalNAc-T14 protein expression between breast carcinomas and non-malignant tissue.

Minor points:

a) Whether GalNAc-T14 could be used as a marker for breast cancer (title) does not seem to be the major goal of this work (though comparison of GalNAc-T14 expression data was done with the typical clinico-pathological factors that are used to describe the type of breast cancer, GalNAc-T14 expression values were not compared with other
known markers of breast cancer) – therefore I suggest to rather focus the title on GalNAc-T14 being another feature of breast cancer cells detected by IHC.

There are many IHC markers to identify breast cancer as such so the authors should find another title.

Response

We amended the title. New title is “N-Acetylgalactosaminyltransferase-14 as a potential Biomarker for Breast Cancer by Immunohistochemistry”.

b) Also please comment on why patients with tumors showing anti-Her2 ++reactivity were considered to be Her2-positive. Usually, at Her2++ protein score, Her2 FISH analysis is done to confirm the positive Her2 status.

Response

ErbB2 staining being classified as positive here displayed strong staining, reflecting gene amplification. In clinical practice, Her2 FISH analysis is done to confirm the positive Her2 status.

c) In the chapter “Methods - Collection of tissue samples…” in line 8 it is
not clear where the normal tissue is coming from. How can there be 16 normal tissue specimen from 5 DCIS patients?

**Response**

I am sorry that it is not described clearly here. These normal tissues which were more than 5cm distant from carcinoma were from the same 23 invasive ductal carcinomas patients. We amended the describing in the chapter “Methods - Collection of tissue samples…”

d) I suggest to shorten table 3 by summarising the individual cases just as it is done in the text. “N” is not explained (does it mean nodal status?).

**Response**

Table 3 was shorten.

e) In the discussion, the authors listed the MCF-7 cell line in the group of aggressive, metastatic breast cancer cell lines. However, MCF-7 cells are rather not aggressive and not metastatic.

**Response**

The sentence that “The cells were chosen to represent a range of phenotypes from 'normal'/benign (HMT 3522), primary, non-metastatic
breast cancer (BT 474), to aggressive, metastatic breast cancer (ZR75-1, T47D, MCF-7, DU 4,475).” was cited from ref [35].

f) At the end of the discussion, the authors should discuss why they consider GalNAc-T14 being a marker for aggressiveness, although this protein seems to be more frequently expressed in G1 tumours than in G3 tumours.

Response

A discussion on a relationship between GalNAc-T14 expression and aggressiveness was added to in the section.

g) The authors may comment on the potential use of the marker for prognosis, prediction and therapeutical interventions since this is described in other cancer entities. There is a need for evaluation of the marker with long-term outcome of the patients.

Response

It was considered as a potential biomarker for breast cancer by immunochemistry according to our results. As you said, it is necessary for evaluation of the marker with long-term outcome of the patients.
h) There are some errors in English that should be corrected.

Response

The errors in English have been corrected.

i) The abbreviation for the enzyme’s name is not consistent throughout the manuscript.

Response

All the abbreviation for the enzyme’s name is edited to GalNAc-T14 throughout the manuscript.
Referee II

1) Quantification of data by computer methods is needed.

Response

Each sample was evaluated by four experienced pathologists. Moreover, the data were analyzed primarily using image processing, enhancement, and analysis software Image Pro Plus 6.0.

2) The authors claimed that GalNAc-T14 is a potential immunohistochemical marker associated with histological grade in breast carcinoma and tumor progression and that the enzyme has a significant association with invasive ductal type, mucinous adenocarcinoma and DCIS type carcinoma. There is no statistic applied to conclude this statement. On the other hand, the manuscript has several contradictions in Results and Conclusions when data is properly analyzed in Tables and Figures. Example: Relationship between the proposed marker and Grade of tumors.

Response

Our data were analyzed using standard statistical software SPSS version 13.0. According to our results, the cases with higher histological grade did show lower levels of GalNAc-T14 expression than the cases with low
histological grade.

3) Figure 1 shows negative normal tissue in relation with the marker, but it seems like the authors did not use the antibody.

**Response**

We did use the nonspecific immunoglobulin as a negative control in Figure1B. In our revised manuscript, Figure1B was replaced with another negative normal tissue.

4) Figure 2B shows negative lobular carcinoma. It is a contradiction with conclusions and Tables.

**Response**

Four invasive lobular carcinomas were evaluated in our study, and 3 of which (75%) were GalNAc-T14 negative, while 1 was immunostained weakly. So we showed the negative lobular carcinoma image.

5) Figure 3: A, B and C. It is not clear whether the marker decreased or increased with malignancy or types of cancer.

**Response**
Figure 3 B was taken a picture using a micro-imaging system different from with which Figure 3 A, C was taken pictures in our former manuscript. In the revised manuscript, Figure 3 A B C were taken photos again using the same micro-imaging system. Figure 3A showed that the case of invasive ductal carcinoma with histological grade 1 was strong GalNAc-T14 immunostaining. Because there was a small quantity of cancer cells in the case of histological grade 1, it did not look like obviously strong immunostaining. In fact, figure 3A did show strong staining in the cancer cells.

6) Confusing results

Capitals letters are missing in several places in Tables. There is no consistency in this regard as well as spaces between words and the title.

Response

The mistakes have been amended.

7) Table 1 Tumor stage

No results

Response

I am sorry for my carelessness. Table 1 has been amended in the revised
8) Table 2 P value 0.00000: It is not clear

**Response**

In fact, it is zero by SPSS.

9) Table 3 N

Nothing under that line

**Response**

Table 3 was shorten in the revised manuscript.