Reviewer's report

Title: N-Acetylgalactosaminyltransferase-14 as a potential New Immunohistochemical Marker for Breast Cancer

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Reviewer: Eva Johanna Kantelhardt

Reviewer's report:

Wu et al present an original paper about immunohistochemical detection of GalNAc-T14 in breast cancer compared to normal breast tissue. This member of the mucin O-glycosylation family has been identified 2003. To my knowledge, there is no work published about the expression in breast cancer so far. Since the enzyme family is known to be involved in tumour progression and also shows predictive value in other cancer entities, the topic is newsworthy and of high interest to oncologists. The correlation between immunohistochemical staining of GalNAc-T14 and breast cancer/normal breast tissue is explored. The pictures of the staining are very nice, the results seem reliable. However, some issues need to be addressed before publication of the manuscript can be recommended.

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.

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.

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.

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.

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?
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?).

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.

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.

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.

h) There are some errors in English that should be corrected.

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

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

**Quality of written English:** Needs some language corrections before being published

**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.