Reviewer's report

Title: Histone demethylase GASC1 - a novel prognostic and predictive marker in invasive breast cancer.

Version: 1 Date: 3 August 2012

Reviewer: Jaydutt Vadgama

Reviewer's report:

Comments for the manuscript entitle: Histone demethylase GASC1 - a novel prognostic and predictive marker in invasive breast cancer.

Overall comments:

This interesting study examined the prognostic role of histone demethylase GASC1 (JMJD2C) in women with invasive breast cancer. GASC1 has been identified as putative oncogene that demethylates tri- and dimethylated lysine 9 on histone H3 (Cloos et al., Nature 2006). GASC1 is mapped on 9p23-24 amplicon. Published data suggest that GASC1 and the jumonji protein family may play an important role in epigenetic signaling, transcriptional regulation and human disease.

Their data suggest that women with GASC1 negative tumors had poor outcome and shorter overall survival. These tumors were mostly of ductal in origin, with high histological grade and more ER/PR negative and HER2 positive (Luminal B?).

The patient cohort consisted of 355 women who enrolled in a Breast Cancer Project in Kuopio, Finland. The GASC1 expression was analyzed by immunohistochemistry and mRNA on tissue microarrays.

Major compulsory revisions:

Two recent studies have clearly demonstrated the biological functions and clinical relevance of GASC1 in breast cancer (Liu et al; Oncogene 2009; and Wu et al; Oncogene 2012). Liu et al (2009) showed that GASC1 as one of the amplified genes for the 9p23-24 region in breast cancer, particularly in basal-like subtypes. The levels of GASC1 transcript expression were significantly higher in aggressive, basal-like breast cancers compared with nonbasal-like breast cancers. In contrast, the current study by Berdel et al., shows GASC1 downregulation is related to poor outcome.

Furthermore, earlier data showed that GASC1 had oncogenic properties and its overexpression was more linked with basal-like phenotypes. In contrast the current study suggests GASC1 negativity is associated with tumors of more aggressive histopathological types (ductal type, grade II and III, ER negative, PR negative). The authors attribute these differences between in vitro cell lines (Liu
et al., 2009) and in vivo clinical samples in this study. However, Liu et al. had also examined tumor tissues and showed data consistent to their in vitro data.

In contrast, others have shown that overexpression of GASC1, a histone demethylase acts as an oncogene and induces transformed phenotypes, including growth factor-independent proliferation, anchorage-independent growth, altered morphogenesis in Matrigel, and mammosphere forming ability. Some studies have suggested that GASC1 demethylase activity may be linked to the stem cell phenotypes in breast cancer. The authors in the current study did not adequately explain the contrasting data.

In addition, the current study did not comment on GASC1 overexpression and demethylation of H3K9me3.

GASC1 protein demethylates tri- and dimethylated H3K9 and H3K36 marks (Cloos et al., 2006; Klose and Zhang, 2007; Shi and Whetstine, 2007; Whetstine et al., 2006). Demethylation of H3K9 activates transcription and loss of H3K9 methyltransferase activity is likely associated with many types of tumors.

The current study fails to discuss these findings.

Minor essential revisions:

The questions posed by the authors are well defined, and the methods are well described. The title and abstract clearly and accurately conveys their findings, and the writing is acceptable with exception to a few typo/grammatical errors made in the paper (noted below). The data is sound, with exception to comments listed below. The conclusions are solid, but the discussion was found to be lacking and does not adequately justify contrasting data.

With regards to data, discussion of limitations and discussion:

- Figure 7: The error bar for relative GASC1 mRNA expression in Grade III samples is large; are results truly statistically significant?
- Table 1: More number of patients with GASC1 overexpression was associated with higher tumor size and the difference was significant. The authors do not provide adequate explanation.
- Table 2: Tumor size (T2, T3 and T4) and Clinical Stage (II, III and IV) has no effect on breast cancer specific survival and time to relapse – counter intuitive? Is this possibly due to the small number of patients with T3 and T4 tumors and at Stage III and Stage IV? If so, this limitation should be addressed.
- In the discussion section, it was mentioned that GASC1 demethylase activity has been possibly linked to the stem cell phenotype in breast cancer, and that this function (of GASC1) might be responsible for a lower recurrence rate in GASC1 positive cases compared with GASC1 negative and better outcome of GASC1 positive patients treated with radiotherapy. My question is if GASC1 functions to maintain a stem cell phenotype in breast cancer, why would you expect the GASC1 positive cases to have a better outcome than the negative cases? Cancer stem cells are thought to be responsible for recurrence of cancer.
Statement seems counterintuitive.

Discretionary Revisions

- Figure 6: Could possibly increase the number of patient samples analyzed for GASC1 mRNA expression levels, and analyze breast cancer specific survival. Too few samples (20 for high GASC1 mRNA expression levels & 37 for low GASC1 mRNA expression levels) were utilized in that analysis.

Minor Essential Revisions

- Figure 1. The images of positive and negative stains for epithelial carcinoma cells should be at the same magnification (either 100x or 200x magnification)

- Page 9 under segment GASC1 mRNA expression is in line with immunohistochemical data – Change statement, “In contrast, HER2 negative cases showed significantly higher GASC1 mRNA expression than HER2 positive ones, which was in line with the protein staining results”.

- Figure 7 and Figure 8: Should include mean expression value for each category in figure legend (histological grade, PR weak/neg, PR strong, HER2-, HER2+).

- Page 11 – correct spelling error – demetylation to demethylation

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

No to all of the above