Reviewer's report

Title: Reevaluation of the 22-1-1 antibody and its putative antigen, EBAG9/RCAS1, as a tumor marker

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Reviewer: SATOSHI INOUE

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General
The authors compared expression of the 22-1-1 and RCAS1-defined antigens in normal and neoplastic tissues. The data suggest that the antigens recognized by the 22-1-1 and an antibody against a recombinant protein encoded by RCAS1 cDNA were different. Although they have already reported that the RCAS1 encoded protein is localized in the Golgi apparatus, the present results include interesting points concerning more precise subcellular distribution characterized by electron and confocal microscopies. Still, RCAS1 expression and clinical prognosis are correlated in several studies using detection for not the 22-1-1-defined antigen but the RCAS1-defined antigen. Thus, the RCAS1-defined antigen could be deregulated and involved in tumors indeed. Reevaluation of previous reports and proposed roles of RCAS1 in tumors should be required.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The major concern of this manuscript is the confusion of term usage ‘EBAG9’ and ‘RCAS1’. In page 5, line 13; the authors refer to the term EBAG9. However, most reports that they contradicted use the term RCAS1. Therefore, the authors should use the term RCAS1 instead of EBAG9 in this manuscript. Indeed, using the term RCAS1, they previously showed that the monoclonal antibody 22-1-1 failed to recognize the RCAS1 protein and the 22-1-1 epitope was identical with the tumor-associated O-linked glycan Tn (ref. 10). Especially, the first paragraph in the Discussion part in p. 15 is the statement for RCAS1 and not for EBAG9 at all. It is the claim of Nakashima et al. (ref. 3) that the 22-1-1 epitope is the RCAS1 protein that induces apoptotic cell death. Although Nakashima et al. isolated the RCAS1 cDNA through an expression cloning using the 22-1-1 antibody, the 22-1-1 epitope is a 78-kDa antigen (ref. 2) whereas the product encoded by the RCAS1 cDNA is detected as a 32-kDa protein (ref. 10). Taken together, they should use only the term RCAS1 in the title and text of their revised manuscript.

Another concern is their negative evaluation of RCAS1-defined antigen as a tumor-specific marker. They described that the immunohistochemical staining by the monoclonal Ab-1 antibody was ubiquitously observed in all the normal and adenocarcinoma tissues that they examined. They also detected 22-1-1 expression in normal cells. Thus, the sentence “In contrast to 22-1-1 staining, ....” (in page 3, line 20) is not correct. Their present results are not systematic pathological studies using a number of carcinoma tissues with different tumor stages. The results in previous literature showing significant correlations between the expression levels of the RCAS1-defined antigen and the cancer progression (ref. 8, 16, and 17) suggest the involvement of RCAS1 deregulation and function in tumors. Therefore, the authors should avoid evaluations of quantitative and tumor-specific roles of the RCAS1-defined antigen in tumors from the present data.

In regard to the usage of antibodies against the RCAS1 encoded protein, they performed immunohistochemistry only by the Ab-1 antibody in comparison to the 22-1-1 antibody whereas Western blots only by the polyclonal antibody. Because this report disagrees with previous RCAS1
reports, they should perform all the experiments using both antibodies before publication.

In figure 4, the authors should compare the data with a N-terminal GFP-tagged EBAG9 construct. They also need to show endogenous RCAS1 protein detected by both monoclonal and polyclonal antibodies against RCAS1.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. In page 12, line 18; “shows that after…..” need be corrected.

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.