Reviewer's report

Title: PI3K Activation is Associated with Intracellular Sodium/Iodide Symporter Protein Expression in Breast Cancer

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Reviewer: Takahiko Kogai

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General
The majority of breast cancer express sodium iodide symporter (NIS), although its expression has been observed mainly in cytoplasm, so it is not functional for iodide transport. In this manuscript, Knostman et al describe post-translational NIS regulation by phosphoinositide-3-kinase (PI3K) activation in MCF-7 breast cancer cells transfected with a constitutively active mutant of p110, a catalytic subunit of PI3K. Its over-expression significantly reduced the NIS expression on the cell surface membrane as well as the induction of NIS by a combination of all-trans retinoic acid and hydrocortisone (tRAH). A correlation between phosphorylated Akt (pAkt) expression and intracellular NIS expression in human breast cancer tissues was also described.

This is potentially a novel finding of the post-translational regulation of NIS in breast cancer. However, there are some issues in data presentation and discussion that should be addressed.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1. Figure 1B clearly demonstrates the absence of NIS on the cell surface membrane in the PI3K activated MCF-7, while Figure 1C does not seem to show a robust difference between wild type and PI3K activated cells. In the right panel, NIS staining is apparent on the cell surface, although the morphology is different from the cells in the left panel. How were the fields selected? Additional photos should be shown.

2. NIS in breast cancer tissues has been reported as be a 70 to 100 kDa protein in both human (Breast Cancer Res Treat 77:157-65) and rodent models (Nat Med 6:871-8). The authors report that up-regulation of PI3K, which is common in breast cancer, generates a 50 kDa NIS (Figures 1 and 2). The discrepancy with published data should be discussed.

3. Cells stably transfected with empty vector (pcDNA3) should be compared as a control with PI3K transfected cells in iodide uptake (Figure 2B) as well as Western blot analysis (Figure 1A). This will avoid artifact(s) by G418 selection and/or the insertion of exogenous DNA to genomic DNA, although these factors unlikely affect to the endogenous NIS expression.

4. Up to 50% of decreased function of deglycosylated NIS has been reported with mutagenesis of NIS at the glycosylation sites (J Biol Chem 273: 22657-63). Are there any other mechanisms to reduce the NIS trafficking with PI3K activation? The contribution of reduced NIS function/trafficking due to deglycosylation should be discussed.

5. Since a correlation between nuclear pAkt and intracellular NIS is suggested in immunohistochemistry (p.13, line 18), representative staining of the pAkt and NIS should be shown.

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. The method for densitometry in Figure 2 should be described.

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential 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, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.