Re: The Role of SATB1 in Breast Cancer Pathogenesis

Iorns et al. (1) concluded that *SATB1* has no role in breast cancer pathogenesis, contradicting our conclusion in Han et al. (2). Although on the surface it appears that both studies (1,2) used similar methods and cell lines, their experiments differ in several critical ways. In addition to the very unclear images (particularly three-dimensional images), their article suffers from lack of critical controls and attention to important details.

1. MDA-MB-231 cells from the American Type Culture Collection are heterogeneous and at times the majority of cells are not aggressive. Thus, when these cells do not behave aggressively, they need to be cloned for aggressiveness (3). Iorns et al. used parental MDA-MB-231 cells and MDA-MB-231 cells containing the control short hairpin (sh) RNA vector that, after tail vein injection, formed metastatic lung nodules in only two of 12 mice (20 nodules in one and 60 in the other); the remaining 10 mice developed from zero to five metastatic nodules per lung [supplementary figure 2D (1)]. In contrast, we injected the same number of cells (our cells were not cloned for aggressiveness) and observed more than 100 nodules in all six control shRNA mice in that experiment [figure 3A (2)]. Therefore, Iorns et al. do not appear to have used aggressive MDA-MB-231 cells for their study.

2. In the wound-healing assay, their so-called "aggressive" MDA-MB-231 cells had not migrated by 36 hours [figure 1D (1)]. Contrast this result with their observation that nonaggressive (parental) MCF7 cells migrated as early as 14 hours [supplementary figure 3D (1)] and formed tumors in mice (apparently without estrogen supplementation) more efficiently than MDA-MB-231 cells [figure 2A and supplementary figure 5A (1)]. These data, and those in point 1, are inconsistent with their own classification of "aggressive" and "nonaggressive" cells, as well as those in the literature.

3. Iorns et al. claim that SATB1 knockdown has no effect on BT549 colony morphology [supplementary figure 1C (1)]. However, BT549 cells have an invasive stellate morphology when grown on Matrigel, and we showed that knockdown of SATB1 in BT549 cells changed their morphology from stellate to spherical [supplementary figure 2B (2)]. Even before SATB1 knockdown, the morphology of BT549 cells in Iorns et al. was similar to that of nontumorigenic MCF10A cells and to our BT549 cells after SATB1 knockdown.

4. To demonstrate that SATB1 knockdown reverses metastatic activity and tumor growth, it is crucial to use isolated clones that are selected for greatly reduced SATB1 transcript levels. In our study, SATB1 knockdown was apparently highly efficient in both the pooled population and isolated cell clones [figures 2A and 3A and B (2)]. To assess experimental metastasis to the lung, Iorns et al. used G418-selected MDA-MB-231 cell populations that would have included cells that escaped efficient SATB1 knockdown and thus could form lung metastasis. Furthermore, because their control cells did not behave aggressively in experimental metastasis, the authors observed "sporadic" (random) patterns of metastasis with both SATB1 knockdown and control cells [supplementary figure 2D (1)]. In contrast, our intra- and extravasation experiments gave completely consistent data [figures 3A and 4B (2)].

5. Because the number of control mice with significant lung nodules (>20 nodules) was low for MDA-MB-231 cells [see point 1, two of the 12 parental and control shRNA mice (approximately 16.7%) (1)], compared with our published data of six of the six control shRNA mice [>100 nodules each (2)], to measure the 84% reduction in lung metastasis that we reported (2) with SATB1-shRNA [two mice with >20 nodules of 12 SATB1-shRNA mice (16.7%) (2)], 85 animals for each group would be required to achieve a reliable conclusion (ie, statistical difference between 16% initial metastatic lung incidence and 84% reduction: $(1 - 0.84) \times 16.7\% = 2.7\%$; χ^2 power analysis with $\alpha = .05$ and power = 0.8, http://www.biomath.info/power/chsq.htm). Thus, they tested too few mice to reach such a conclusion.

6. High levels of exogenous overexpression of SATB1 in many cells, including SKBR3, makes them susceptible to apoptosis with passage (H. Han, Y. Kohwi, and T. Kohwi-Shigematsu, unpublished data). For cell selection, Iorns et al. used a higher concentration of G418 (1.5 mg/mL) than the one that we used (0.6 mg/mL). Our SATB1-expressing cells cannot survive selection in G418 at 1.5 mg/mL, unlike those used by Iorns et al. Nevertheless, it is likely that those cells expressing particularly high levels of SATB1 required for tumor formation were low in number or eliminated, after selection in G418 at 1.5 mg/mL. It is also worth mentioning that, for our tumor formation studies, we used a pool of more than 40 individual clones that were verified for high SATB1 expression (with minimum cell passage). Thus, we suggest that their SKBR3(pLXSN-SATB1) cells did not form tumors because their sample likely contained too few critical high SATB1-expressing cells.

7. Iorns et al. reported that SATB1 transcript levels are unchanged between aggressive and nonaggressive beast cancer cell lines in microarray datasets available online [figure 5 (1)]. However, the probe sequences that they used for hybridization studies are not SATB1 specific—that is, the probes overlap with the SATB2 sequence in eight to 11 of 25 nucleotides [supplementary table 3 (1)]. The same problem exists for evaluating SATB1 mRNA levels in patient samples in published datasets [figure 6 (1)]. By quantitative polymerase chain reaction with SATB1-specific primers (2) and an alternative 5′-GGAGCCGTT CTTGGTTTCA-3′ (forward) and 5′-TT AGACATTTCTGAATGTTC-3′ (reverse) primer set, we have confirmed our published data [as shown in figure 1A (2)] (E. Ordinario, H. Han, Y. Kohwi, and T. Kohwi-Shigematsu, unpublished results).

8. Results supporting the conclusions by Han et al. include 1) SATB1-dependent invasive and morphological behaviors in a number of cell lines have been independently reproduced (M. J. Bissell, personal communication), 2) SATB1 expression is greatly increased in multidrug-resistant

breast cancer cells with high invasive potential (4), and 3) high SATB1 expression is reported in human tumors with advanced stages of breast cancer (5) and laryngeal squamous carcinoma (6).

9. Finally, and most importantly, the association between SATB1 expression and prognosis cannot be assessed by only its transcript levels because SATB1 expression is not limited to tumor cells. Activated stromal cells also express SATB1. Consequently, immunostaining must be used to show definitively whether individual tumor cell express SATB1 protein in their nuclei. Unlike Iorns et al., who based their conclusion only on publicly available RNA datasets from other groups [figure 6 (1)], we used immunostaining to score SATB1 expression in tumor cells in more than 1000 human breast tumor specimens [figure 1D and supplementary figure 1B (2)].

Recently, Lu et al. (7) reported that high SATB1 levels correlate with poor prognosis in human gastric cancer.

TERUMI KOHWI-SHIGEMATSU
HYE-JUNG HAN
JOSE RUSSO
YOSHINORI KOHWI

References

1. Iorns E, Hnatyszyn HJ, Seo P, Clarke J, Ward T, Lippman M. The role of SATB1 in breast cancer pathogenesis. *J Natl Cancer Inst.* 2010; 102(16):1284–1296.
2. Han HJ, Russo J, Kohwi Y, Kohwi-Shigematsu T. SATB1 reprogrammes gene expression to promote breast tumour growth and metastasis. *Nature.* 2008;452(7184):187–193.
3. Minn AJ, Gupta GP, Siegel PM, et al. Genes that mediate breast cancer metastasis to lung. *Nature.* 2005;436(7050):518–524.
4. Li QQ, Chen ZQ, Xu JD, et al. Overexpression and involvement of special AT-rich sequence binding protein 1 in multidrug resistance in human breast carcinoma cells. *Cancer Sci.* 2010;101(1):80–86.
5. Patani N, Jiang W, Mansel R, et al. The mRNA expression of SATB1 and SATB2 in human breast cancer. *Cancer Cell Int.* 2009;9:18.
6. Zhao XD, Ji WY, Zhang W, et al. Overexpression of SATB1 in laryngeal squamous cell carcinoma. *ORL J Otorhinolaryngol Relat Spec.* 2010;72(1):1–5.
7. Lu X, Cheng C, Zhu S, et al. SATB1 is an independent prognostic marker for gastric cancer in a Chinese population. *Oncol Rep.* 2010;24:(4):981–987.

Funding

Verification experiments and preparation of this correspondence were supported by funding from the National Cancer Institute R37 CA39681 grant.

Notes

The MDA-MB-231, BT549, and SKBR3 cells, described above, were authenticated by American Type Culture Collection by karyotyping and isoenzyme analysis. Date of this authentication are undetermined.

The authors had full responsibility for the design of the study, the collection of the data, the analysis and interpretation of the data, the decision to submit the manuscript for publication, and the writing of the manuscript.

Affiliations of authors: Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720 (TK-S, H-JH, YK); Breast Cancer Research Laboratory, Fox Chase Cancer Center, Philadelphia, PA (JR).

Correspondence to: Terumi Kohwi-Shigematsu, PhD, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720 (e-mail: tkohwi-shigematsu@lbl.gov).

DOI: 10.1093/jnci/djq440

Advance Access publication on November 12, 2010.