Reviewer’s report

Title: Chemotherapy induces Notch1-dependent MRP1 up-regulation, inhibition of which sensitizes breast cancer cells to chemotherapy

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Reviewer: William T Beck

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Drs. Kim et al have tested the hypothesis that Notch 1 regulates the ABCC1 transporter, MRP1, and causes drug resistance in some breast cancers. They base the hypothesis on two independent observations: (i) one study showed that MRP1 was a direct transcriptional target of Notch1, and (ii) the other showed that Notch signaling was upregulated in breast tumors of patients who had been treated with neoadjuvant chemotherapy (NAC).

Accordingly, the authors did immunostaining for these two proteins in breast tumors from 29 patients and found that these proteins increased after NAC and their increase appeared to be related. To better understand this phenomenon, the authors examined expression of these genes/proteins in two cell lines (T47D BC and HB2, an immortalized but not transformed line derived from human breast) following various manipulations. The present data indicate that treatment of cells with doxorubicin (DOX) increased ABCC1 and MRP1 in both lines, but increased Notch1 only in the T47D cells. This is curious because they show that the targets of Notch1, Hes1 and Hey1, are increased in both cell lines. Increased MRP1 was apparently associated with increased efflux of calcein and DOX, and inhibition of Notch1 by DAPT could impair this efflux. Finally, inhibition of Notch1 expression by N1-specific siRNA decreased ABCC1 mRNA only in the T47D cells.

While the hypothesis is compelling, the patient tumor data supportive, and the general methods acceptable, there are some concerns that diminish enthusiasm for this manuscript in its present form. These include the descriptive nature of the study and lack of mechanistic underpinning, the use of only one transformed and one untransformed cell line, and as a consequence, over-reaching and unjustified speculation and interpretation of the data. Specifically,

1. The work is largely descriptive, with no mechanistic underpinning.
2. While the in vitro data with the T47D cells support the hypothesis and the patient tumor data, those for the non-transformed HB2 cells are somewhat problematic. This could be a function of the fact that the authors only examined two cell lines. It seems to this reviewer that examination of several more transformed and non-transformed breast cell lines is necessary to provide assurance that this disconnect in the non-transformed lines is real.
3. Further, the data were only a “snapshot” in time. Had the authors looked at
expression of Notch1 and MRP1 in these cell lines at different times after the various pharmacologic manipulations, they might have obtained different results.

4. Because of the problems with only the two cell lines, the speculations in Fig 7 as well as the conclusions on lines 429-432 are not warranted at this time.

Other concerns:

5. Table 1 and lines 265-266: The lack of significant correlation of Notch1 and MRP1 with clinical or pathological features is possibly a consequence of the small sample size. No conclusions can be made about clinical or pathological relationships with this dataset.

6. Fig 3A and line 278: the western blot is of very poor quality.

7. Lines 278 and 310: Expression of MRP1 may have been increased by treatment with DOX, but the applicants cannot state unequivocally that MRP1 expression was “induced”. 

8. Fig 3C and lines 286-288: while the differences in efflux may have been statistically significant, one has to ask if the data represent biological significance, as the differences appear to be quite small.

9. Fig 6C and lines 352-353: synergism has a specific biologic and pharmacologic meaning. The authors cannot state that the DAPT – DOX combination was “synergistic” unless they perform a Combination Index or Isobologram analysis.

10. Lines 357-364: because of the concerns in #1 – 3, above, the speculation that DOX-induced up-regulation (increased expression would be a better term) of MRP1 is independent of Notch1 signaling in HB2 cells is not warranted.

11. Lines 382-383: “implicating” … MRP1 in … resistance”. There is a clear association, but it is far from clear that MRP1 is implicated in this clinical phenomenon; it is likely much more complex.

Overall, this is an interesting study, based on a good hypothesis and compelling, but limited data from the breast tumors of a small number of patients. The cell line data are deficient in that they are derived from one transformed and one non-transformed cell line, at only one time point (except for the efflux studies), and the speculations based on these limited data are not justified at present. In addition to the limited ability to understand the phenomenon with two cell lines, the study lacks mechanistic underpinning and is largely descriptive with no molecular manipulations to help understand this interesting association between Notch1 and MRP1.

**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