Reviewer’s report

Title: TOX3 is Expressed in Mammary ER+ Epithelial Cells and Regulates ER Target Genes in Luminal Breast Cancer

Version: 2 Date: 25 October 2014

Reviewer: Ana-Teresa Maia

Reviewer’s report:

The authors present an expression analysis of TOX3, both gene and protein, in breast cancer samples. They have investigated in particular subpopulations of cells, and subsets of tumours. They perform studies in human and murine tissues.

They report expression of TOX3 in ER+ luminal epithelial cells, and relate high expression with poor outcome in luminal B tumours. A knockdown experiment also suggests slower cell growth.

Finally they suggest that TOX3 might have a dual role in initiation and progression of tumours.

- Major Compulsory Revisions
  1 - Throughout the manuscript both the gene and the protein are written as “TOX3”. It is extremely confusing and unnecessarily, as genes should be written in italics, and thus distinguish immediately from proteins. Authors should clarify the text in this respect.

  2 – In the Background section the authors have a very succinct phrase about risk association with tumour sub-type, particular when so much has been reported. This aspect should be described better and referenced. Nordgard et al (Breast Cancer Research 2007, 9:113) report an up-regulation of TOX3 in luminal A, luminal B and ErbB2+subtypes and down-regulated in the basal-like subtype. Additionally they report an over-representation of the minor allele of rs9940048 in the basal-like subtype, consistent with low levels of TOX3 mRNA in these tumours. Tapper et al (Breast Cancer Research 2008, 10:R108) report an association between the rare risk allele rs1420542 with distant metastases, as well as an association with ER+ tumours. A weak, but significant association wit ER- tumours had been found by Garcia-Closas et al (PLoS Genet. 2008 Apr 25;4(4):e1000054).

  3 - The authors mention a report that proposes TOX3 as a TSG, but in fact there has been a mutation screen report, Jones et al (PLoS One. 2013 Sep 19;8(9):e74102), which provides stronger support for this role of TOX3, and should be referenced in the background and Conclusions sections. Additionally, TOX3 mutations seemed to be associated with Luminal A and ER+ tumours.

  4 – Figure 1B shows “Relative Tox3 expression” in sorted cell populations. It is
not clear what these are in relation to. Nevertheless, although there seems to be a significant difference between luminal and basal cell populations, the use of the expression “highly expressed” in line 181 of the manuscript is inappropriate. It should read “more expressed”.

5 – When in line 218 the authors write that the ratio of the two forms is similar between breast cancer cell lines and normal samples, this is not very clear from Figure2B. Var2 does not seem to be expressed in either normal samples. In fact, it shows that TOX3 expression is very low in normal tissue, as previously described.

6 – The authors present contradictory results when in Figure 3B they show that LumB tumours have a higher % of TOX3 expression, and in Figure2C it is clear that LumA and mApo tumours have higher expression TOX3. Taking this into consideration, I do no think that one can suggest TOX3 expression as a marker for LumB tumours.

7 – the authors attribute a transcriptional activator role to TOX3, nevertheless, they only detect gene expression changes upon transient transfection of TOX3. The gene expression changes could be indirect. To prove transcriptional role TOX3 should be shown to bind to regulatory elements of those genes, or the candidate regions where TOX3 could bind, should be shown to have promoter/enhancer potential in in vitro studies.

8 – since the authors have access to microarray data from tumours, they should show that the genes they observe to be up/downregulated upon transfection of TOX3, have the same behaviour in the different subtypes of cancer, depending on their TOX3 expression level.

- Minor Essential Revisions

1 - Line 418 – “Nevertheless, unbound ER has recently been shown to…”

- Discretionary 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'