Reviewer's report

Title: The Wnt signalling pathway is upregulated in acquired Tamoxifen resistant breast cancer.

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Reviewer: Elizabeth Vincan

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Using a laboratory established in vitro culture system; Loh and colleagues provide compelling evidence for a causative role for Wnt signalling in the acquired resistance of breast cancer cells to tamoxifen. Clinically, acquired resistance to tamoxifen is accompanied by detectable changes in the cancer cells; they become more aggressive and metastatic, and these phenotype changes are mirrored in this in vitro model. The study demonstrates increased canonical (β-catenin dependent) and non-canonical (β-catenin independent) Wnt signalling in tamoxifen resistant cells. This increased Wnt signalling was accompanied by concomitant increases in the expression of genes associated with a more aggressive and invasive phenotype (EMT genes) in the tamoxifen resistant cells. This paper makes an important contribution to our understanding of the mechanisms underlying drug resistance in breast cancer cell but would benefit from some necessary revision.

Major compulsory revision:

1. The data clearly indicate that the non-canonical Wnt pathways are up-regulated in the tamoxifen resistant cells, even more so than the canonical pathway. That is, the largest increases in gene expression are Ror2 and JUN, which are non-canonical Wnt pathway components and strongly indicate an increase in Wnt/PCP signalling. Secondly, DKK, which primarily inhibits canonical Wnt signalling as it binds to LRP, is also dramatically increased in the resistant cells. The increase in Wnt/PCP components and an inhibitor of canonical signalling should be highlighted – this is a very interesting finding. An increase in canonical Wnt signalling is none the less evident (significant increases in reporter activity and c-myc expression etc) but I would conclude that canonical pathway is possibly constrained (by increases in DKK) in the resistant cells. Also, the increase in AXIN2 is relatively small (not sure if it is significant as Fig 2A does not have a * for AXIN2).

Near the end of the discussion there is reference to both flavours of Wnt signalling being up regulated, which is clearly supported by the data, however in other sections of the paper interpretation of the results is more single minded, focusing on the canonical pathway. For example, PORCN facilitates secretion of all Wnt glycoproteins yet it is referred to as “….support the activation of the canonical Wnt pathway in response to Tamoxifen.” in the discussion. The increase in CSNK1A1 does indicate increased canonical signalling but increased
PORCN does not. Careful revising to ensure only bona fide Wnt target genes are referred to as Wnt target genes, and as already mentioned, canonical vs. non-canonical roles are clarified, as supported by the data, is required. There is emerging evidence that the non-canonical Wnt pathways are involved in tumour invasion, so this is a very timely article as it indicates that the Wnt/PCP pathway might be involved in tamoxifen resistance and the acquisition of migratory and invasive phenotype in breast cancer.

Minor essential revision:
1. Ensure that µ (not u) throughout.
2. If increase in AXIN2 expression in Fig 2A is not significant (no *) then don’t overstate the results – trend towards increase is fine. AXIN2 is considered a global Wnt target gene, but there are instances where increases are small, so that’s fine.
3. Add * for significance in Fig 3C.
4. As Mentioned above, PORCN is necessary for Wnt secretion, thus inhibition of PORCN is a global inhibition of Wnt signalling – not specific for the canonical pathway.

Level of interest: An article of importance in its field

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