Reviewer’s report

Title: Loss of Akt1 or Akt2 inhibits mammary tumor onset and growth in vivo

Version: 2 Date: 17 June 2013

Reviewer: Fekicity EB May

Reviewer’s report:

This article reports the authors' investigation of the importance of Akt1 and Akt2 in the development of IGF-1R-induced mammary tumors in a mouse model. The authors have evaluated the phenotypes of the tumors that arise in the different mice with care. The authors present some potentially interesting results. There are problems with the presentation and interpretation of the results to be addressed. The authors should demonstrate restraint and acknowledge more conscientiously the experimental context of their results and conclusions.

Major compulsory revisions.

1. Please alter the title to reflect the content of the study. Loss of Akt1 or Akt2 inhibits IGF-1R-induced mammary tumor onset and growth in vivo.

2. Please ensure that the context of the study and conclusions are stated clearly in the abstract and throughout the manuscript.

3. Please clarify what is meant by: ‘Therefore, inhibitors targeting Akt activation should inhibit breast cancer growth’.

4. Please reduce repetition, the conclusions at the end of the abstract and the discussion are identical.

5. The first figure compares expression or protein phosphorylation in mammary tissue from wild-type mice and in tumor tissue from IGF-1R transgenic mice. The comparison should be between mammary tissue in wild-type and IGF-1R transgenic. Because the authors wish to compare the effects of expression of IGF-1R on the expression or protein phosphorylation, the comparison should not be between normal and tumor tissue. The images for Akt1, Akt 2 and Akt 3 should be replaced with images of better quality. Please explain why there are three protein bands for IGF-1R. There is only one protein band for phosphorylated IGF-1R. Akt3 levels are not much lower that Akt2 levels. Have the authors designed their experimental approach based on the results presented or because the literature suggests that Akt1 and Akt2 are more likely to have a significant role in IGF-1R-induced mammary tumors?

6. Please make the x-axes the same scale in figures 2A and 2B to facilitate comparison.

7. The fifth figure contains some images that should be replaced with others of better quality, notably pErk in figure 5B. There are air bubbles in three of the five analyses of pAkt for the MTB-IGF-1R/Akt/- mice. The analysis should be
repeated because it will have been compromised by the air bubbles.

What is the scale on the y-axis?

From the raw data it is difficult to understand why there is no difference in the level of pAkt between MTB-IGF-1R and MTB-IGF-1R/Akt1-/- . In contrast given the data presented, it is difficult to understand how the authors have determined that Erk expression is significantly lower in MTB-IGF-1R/Akt1-/- mice compared to in MTB-IGF-1R mice. How are the pStat3 results so high? The results for pErk do not appear as dissimilar between MTB-IGF-1R/Akt1-/- and MTB-IGF-1R/Akt2-/- as is suggested by the histograms; they appear remarkably similar.

8. Immunohistochemical comparison of Ki67 does not constitute determination of proliferation rate. Why is this analysis not included alongside the data shown in figures 6 and 7, which could be combined?

9. Some of the immunohistochemical analyses are of concern. There is strong positivity in the stromal compartment. The analyses should be repeated with higher dilutions of or better antibodies.

10. Please give more details about the SGR and how it is calculated. Please explain given the large overlap between the tumor volume curves for MTB-IGF-1R and MTB-IGF-1R/Akt2-/- mice how the SGRs are so dissimilar. Conclusion ‘the majority of the tumors grew at a slower rate than the mammary tumors of MTB-IGFIR mice’ should be moderated.

**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:**

None to declare