Author's response to reviews

Title: Activation of Akt1 accelerates carcinogen-induced tumorigenesis in mammary gland of virgin and post-lactating transgenic mice

Authors:

Yanyuan Wu MD (yanyuanwu@cdrewu.edu)
Juri Kim BS (jukim@cdrewu.edu)
Yaya Elshimali MD (elshimali@gmail.com)
Jaydutt V Vadgama PhD (jayvadgama@cdrewu.edu)

Version: 3

Date: 13 February 2014

Author's response to reviews:

February 7, 2014
Professor Marcello Maggiolini
Associate Editors
BMC Cancer
Subject: Manuscript MS: 1238098847111914
Title: Activation of Akt1 accelerates carcinogen-induced tumorigenesis in mammary gland of virgin and post-lactating transgenic mice

Dear Professor Maggiolini,

On behalf of my co-authors, I thank you and the Reviewers for BMC Cancer for the opportunity to submit our revised manuscript. We are grateful to the reviewers for their constructive suggestions.

As requested, we have made revisions (explained in details below) and addressed to the best of our ability, the concerns raised by reviewers. We have also followed the journal guidelines for the revised manuscript.

We sincerely thank you and the Editors/Reviewers for the opportunity to publish our interesting study in BMC Cancer.

Please let me know if you need any further information.

Sincerely,

Jay

Dr. Jay Vadgama
Interim Executive Vice President for Research and Health Affairs
Associate Vice President for Research and Health Affairs
Professor of Medicine
Response to Reviewers Comments:

Reviewer #1

Reviewer's report:

Discretionary Revisions:

The results are convincing and confirm the hypothesis, however, considering that Akt system is well established to interact with ER system; the model could be useful to study only some mechanism of ER positive breast tumor development. So, the last sentence in conclusion needs to be revised.

Response: We have modified our conclusion according to Reviewer's suggestion. Please see the conclusion in the abstract and in the main body of the manuscript.

Reviewer #2

Reviewer's report:

Major criticisms

Authors should explain in detail the differences of their model compared to what already published and the relevance of the use of DMBA co-carcinogenetic effects to the human breast cancer.

Response: We are aware that some groups have generated Akt1-transgenic mouse model. In fact we have sited all of their publication and discussed the data obtained from our study and compared to their data in our manuscript. Our design of transgenic mouse model is similar to the models reported by Drs. Schwerfeger and Blanco-Aparicio, using myr-Akt1 sequence which leads to constitutive activation of Akt1 in the plasma membrane under control of MMTV promoter. The model generated from Dr. Ackler’s group used human AKT1 sequence instead myr-Akt1 sequence, even though it resulted elevated pAkt1 in the transgenic mouse. The early studies from Schwerfeger and Ackler et al have showed that mammary gland involution was delayed by activation of Akt1 in
transgenic mice. Recent studies from Blanco-Aparicio et al reported DMBA-inducing ER-positive mammary tumors in myr-Akt1 transgenic mice that provided in vivo evidence of activation of Akt1 associated with ER-positive breast tumor development. PI3K/Akt pathway plays a major role in cancer cell proliferation and anti-cancer drug resistance in different type of cancers including breast cancer. Our group has a long term interest in understanding the role of Akt in tumorigenesis and the development of drug-resistance. We have showed in vitro studies that activation of Akt1 was associated with HER2-overexpressing breast cancer resistance to herceptin and ER-positive breast cancer resistance to Tamoxifen. Our data from clinical studies also demonstrated clearly that activation of Akt1 in breast cancer tissues decreased disease-free survival significantly. Even though the role of Akt1 in tumorigenesis has been investigated by several groups we feel that the mechanisms associated with the oncogenic role of Akt remains to be further elucidated.

The purpose for us to generate this myr-Akt1 transgenic mouse model is to further elucidate the oncogenic role of Akt1 in mammary tumor development. The aim of our study is more toward cancer development and prevention. We asked questions that whether expressing high active Akt1 in mammary tissue would increase the risk to develop breast cancer, or if constitutive activation of Akt1 could accelerate carcinogen-induced mammary tumor development. Since DMBA is known to induce mammary tumor, we also selected to use DMBA. Other studies examined tumorigenesis of Akt1 in transgenic mouse and induced by DMBA in younger virgin mice (starting ages from 6-weeks to 9-weeks). Our study was designed to examine the oncogenic role of Akt1 in both virgin and post-lactating stages, since breastfeeding has been shown to reduce the risk of breast cancer in humans. Our data is in agreement with the data reported by Blanco-Aparicio group that DMBA induced mammary tumors in virgin transgenic and WT mice, but the frequency was higher in transgenic mice. In addition we also showed that post-lactation prevented DMBA induction of mammary tumor in WT mice, but not in transgenic mice. The incidence of DMBA inducing mammary tumor in post-lactating myr-Akt1+ mice was higher than that in virgin transgenic mice. Therefore, it is very worthwhile to report our data to the field of breast cancer research.

2. The authors should include a greater number of mice in most experiments, especially in those that analyze the incidence of malignant and benign lesions in DMBA induced virgin and post-lactating transgenic mice (Table 3).

Response: We have enlarged our number of mice for the study during these months and the number of mice has been increased to 28 per genotype and data reported in Table 3 has been modified according to the new numbers.

Minor criticisms:

The H&E and the IHC analysis in figure 1C that is meant to illustrate the histology and the staining for pAkt are blurry and should be replaced.

Response: Figure 1C has been improved.

In figure 2A the authors measured the estradiol level (mean of two samples plus
SD). They should increase the number of samples to assume that the up-regulation of hormone-response is driven by MMTV-myr-AKT1.

Response: We have increased the number of samples in each group for measurement of estradiol level and figure 2A has been modified.

The authors note a positively association between Akt1 activity and ER alpha nuclear expression. They also observed that cyclin D1 expression is higher in DMBA-induced myr-Akt1 driven mammary carcinomas. These findings have been already reported.

Response: As we mentioned above that although our model is similar to Blanco-Aparicio’s model, but our study design is different than theirs. Their study was focused on examining oncogenic transformation of myr-Akt1 and DMBA increased susceptibility to mammary tumor formation in younger virgin mice and explored that the DMBA-induced mammary tumors were more likely to be ER-positive and had increased cyclinD1 in some of the tumors. Our study has focused on understanding the oncogenic role of myr-Akt1 in different ages and stages of mammary development. At the same time we also confirmed with our model that the myr-Akt1 driven mammary tumors induced by DMBA were more likely to be ER-positive and the expression of cyclin D1 was varied in mammary tumors from transgenic mice but lower in tumors from WT mice. Interestingly we found that the mesenchymal markers, Twist and Slug expression were inversely correlated to cyclin D1 expression. The consistent data reported from different groups using similar model is more convincing.

Reviewer #3
Reviewer’s report:
1. Authors need to show confirmation transgene integration, copy number and also need to provide a transgene map.
Response: An example of the confirmation transgene integration and copy number has been shown in the additional file 2-Additional figure S1. The transgene map has also been demonstrated in the additional file 2-Additional figure S1.

2. Data on how activated Akt1 affect mammary growth, ductal, glandular etc. is missing. Whole mount comparison at different ages in comparison with wild type is needed. Also authors need to discuss how their observations differ with previously published similar in vivo model.
Response: We have performed whole mounts comparison between transgenic and WT mice in different ages and mammary development stages. The data has been shown in the new figure 3. Using similar Akt1-transgenic mouse models Schwerfeger and Blanco-Aparicio groups reported a delayed mammary gland involution in the Akt1-transgenic mice compared to WT mice. According to reviewer’s suggestion we examined mammary gland development in different ages and stages in myr-Akt1+ mice and compared that to WT mice. Our data showed an early adult mammary gland with significant increased numbers of TEBs in myr-Akt1+ mice at age 12-week old compared to WT mice. Similar to other studies the involution was delayed in transgenic mice. The details have
been added into results of manuscript and discussed in the discussion section.

3. Authors provided no discussion on why estradiol levels are higher in transgenic mice compared to wild type.

Response: We have discussed more on the reason(s) for higher estradiol levels in transgenic mice in the discussion section of our revised manuscript. The increased circulating estradiol levels in transgenic mice could be due to the increased Akt levels in ovaries in transgenic mice in our model. Activation of Akt1 in ovaries may increase proliferation of granulosa cells and lead to increase in production of estradiol even in the later-lactating stage. The higher level of estradiol in transgenic mice may also further induce active Akt levels. However, the mammary tumorigenesis could be more driven by the expression of myr-Akt1 in mammary gland since we did not observe any ovary and uterus cancers in our model. Conversely, local estrogen biosynthesis such as regulation of aromatase expression by activation of Akt1 in mammary gland or expressing high level of prolactin induced by myr-Akt1 could all contribute to the mammary tumorigenesis. ###

4. Is there an effect on mammary tumorigenesis or growth in the absence of ovaries? This is critical in light of increased estradiol levels in transgenic mice.

Response: We appreciate the critical scientific and relevant question from the reviewer. We have not examined mammary tumorigenesis in absence of ovaries and could look at this in the future.

5. Figure 3 C needs to show number of animals. Statistical significance and error bars are missing in figures 3 D.

Response: Since we have now included whole mount data as new figure 3, the old figure 3 has been changed to figure 4. The number of animals has been added in the figure 4C and statistical significance has been added in figure 4D. Since the bar graphs in figure 4D represents cumulative incidences of tumors (the numbers are not the mean of different time of DMBA induction), therefore error bars are not needed.