Androgen Receptor driven transcription in molecular apocrine breast cancer is mediated by FoxA1


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Review timeline:
Submission date: 20 May 2011
Editorial Decision: 08 June 2011
Revision received: 08 June 2011
Accepted: 09 June 2011

Transaction Report:
(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 08 June 2011

Thank you very much for transferring your research manuscript including original referee comments and your point-by-point response for consideration to The EMBO Journal editorial office. On this background and upon careful editorial inspection of your work on FoxA1-mediated androgen-receptor driven transcription in apocrine breast cancer, we decided to involve only one trusted referee to enable a timely assessment of your work. As you will see from his/her comments, publication in The EMBO Journal is very much encouraged pending on minor revisions.

Though unavoidable for some points, I kindly ask you to limit further experimentation to facilitate a rapid, final assessment. Please do not hesitate to contact me in case of further questions (preferably via E-mail). Formerly, I also have to remind you that it is EMBO J policy to allow a single round of revisions only and that the ultimate decision on acceptance depends on the content and strength of the final version of your manuscript.

I remain with best regards and look very much forward receiving your revision.

Yours truly,
Editor
The EMBO Journal

REFEEEREE REPORT
Referee #1:

Discerning the molecular mechanisms that underpin distinct breast cancer subtypes is of strong significance to the cancer and nuclear receptor fields. Little is understood with regard to the apocrine subtype, which is characterized as estrogen receptor-alpha (ER) negative but maintains an expression profile reminiscent of ER+ disease. The present study explores the novel and provocative hypothesis that the androgen receptor (AR) may contribute to the apocrine phenotype, as AR-positivity is a hallmark of apocrine tumors. Using a combination of genome-wide analyses and functional analyses it is concluded that: i) apocrine cells are reliant on AR for growth; ii) AR binds sites similar to ER in this cell type, as opposed to observations in prostate; iii) AR binding is concordant with sites of FoxA1 binding; and iv) FoxA1 is required for AR binding and function. Taken together, it is proposed that in the absence of ER, apocrine tumors utilize AR to maintain an ER-like gene expression program.

Critique:

This is a novel study that will be of significant impact to the field. The authors have responded very well to the previous critiques, and added substantive new data to support the stated conclusions.

Addressing the following issues would add to the impact of the findings:

1. Introduction: The statement that "...AR is classically thought of as a driver of prostate cancer" should probably include the additional concept that AR drives prostate cancer development and progression. A citation should be included for the benefit of readers not familiar with the role of AR in prostate cancer.

2. The authors should discuss what models of molecular apocrine disease exist, and therein provide some justification for the use of MDA-MB-453 cells for the study.

3. Given the comparison to LNCaP, the authors should discuss ER status in this cell type.

4. Figure 1F- What is the "AR binding site" amplified in Figure 1F? This experiment needs careful experimental description and justification.

5. Supp Fig 1D- These data appear to lack statistical analyses and do not report error. These issues should be addressed.

6. What is the impact of androgen depletion (for example, as achieved through culture in charcoal-stripped serum) on gene expression and cell growth of MDA-MB-453?

7. Fig 2A- How the cells were treated prior to the ChIP-Seq experiment should be more fully disclosed (eg timing of steroid starvation and hormone stimulation)

8. Similar to the comment regarding 1F, the sites assessed in Figure 3E should be disclosed and justified for selection.

9. Figure 4B would benefit from amplification of an AR binding site not reliant on FoxA1.

10. Fig 4G- The gene identities for the molecular apocrine signature should be annotated for legibility.

11. Fig 4G- What is the impact of siAR on the molecular apocrine signature?

Below are our specific comments to the reviewers concerns.

1. *Introduction: The statement that "...AR is classically thought of as a driver of prostate"*
cancer" should probably include the additional concept that AR drives prostate cancer development and progression. A citation should be included for the benefit of readers not familiar with the role of AR in prostate cancer. This is a valid point and we have now included text and references to address this.

2. The authors should discuss what models of molecular apocrine disease exist, and therein provide some justification for the use of MDA-MB-453 cells for the study. There is very little evidence of any model of molecular apocrine except the MDA-MB-453 cells. We include additional text about this.

3. Given the comparison to LNCaP, the authors should discuss ER status in this cell type. We have now included text addressing this concern. LNCaP cells do not express detectable ER.

4. Figure 1F- What is the "AR binding site" amplified in Figure 1F? This experiment needs careful experimental description and justification. The two regions we picked for this experiment have been previously shown to be AR or ER binding events. We now include the relevant references for these.

5. Supp Fig 1D- These data appear to lack statistical analyses and do not report error. These issues should be addressed. We apologise for this and have now included the statistical analysis for both Supp 1D and Supp 4C.

6. What is the impact of androgen depletion (for example, as achieved through culture in charcoal-stripped serum) on gene expression and cell growth of MDA-MB-453? The cells growth arrest and gene expression is significantly reduced in hormone deprived conditions. All our genomic experiments were conducted in asynchronous cells, avoiding the issues of hormone depletion.

7. Fig 2A- How the cells were treated prior to the ChIP-Seq experiment should be more fully disclosed (eg timing of steroid starvation and hormone stimulation) These cells were not hormone deprived and were grown in asynchronous conditions. This is explained in the manuscript.

8. Similar to the comment regarding 1F, the sites assessed in Figure 3E should be disclosed and justified for selection. We have now included text explaining that this was a region discovered by ChIP-seq.

9. Figure 4B would benefit from amplification of an AR binding site not reliant on FoxA1. This is very difficult to address since no AR binding events are independent of FoxA1. The 98.5% overlap between AR and FoxA1 suggest an absolute dependency. The remaining 1.5% of AR binding events are simply due to threshold issues. As such, there is no AR binding events not reliant on FoxA1.

10. Fig 4G- The gene identities for the molecular apocrine signature should be annotated for legibility. We have now included a table with the gene lists in readable font. This is now included in Supplementary figures.

11. Fig 4G- What is the impact of siAR on the molecular apocrine signature? This is a valid concern, but given time restrictions, we are unable to conduct the siAR transfection followed by microarray analysis. We have instead focused on a subset of molecular apocrine genes which are assessed by qRT-PCR.