Author's response to reviews

Title: Dichotomous roles for the orphan nuclear receptor NURR1 in breast cancer

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The Biomed Central Editorial Team
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We would like to thank the reviewers for lending their time and expertise for the review of this manuscript. The insight provided has been thoroughly considered and has been implemented for the revision of this manuscript where feasible.

Reviewer: Alexander Deutsch

“Needs some language corrections before being published”

In accordance with this recommendation, this manuscript has been reviewed by a 3rd party to correct any grammatical/typographical errors.

Reviewer: Hsin-Hsiung Tai

Discretionary revisions

1. “It would be nice to have some cellular data supporting the change in invasive behavior of breast cancer cells following NURR1 silencing.”

We agree with the reviewer that data linking to NURR1 expression to invasiveness would strengthen this manuscript. As such, we have updated figure 4 to include xenografts from the highly invasive MDA-MB-231 breast cancer cell lines with and without NURR1 silencing, which demonstrates a loss of contralateral metastases with NURR1 silencing, again demonstrating that NURR1 silencing has an effect on tumor progression. The text has also been edited to reflect this addition as indicated below:

“4A2KD-231 cells were more efficient in establishing tumors, and by day 35 showed a substantial increase in growth as compared to Vec-231 derived tumors. Additionally, 4 of 6 mice inoculated with Vec-231 cells developed detectable contralateral mammary lesions whereas none were detected among mice inoculated with 4A2KD-231 cells, highlighting a potential role for NURR1 expression in breast cancer cell invasion.”

2. “The authors indicate that the transcriptional activity of NURR1 may be the key to elucidating its role in the inhibition or promotion of breast cancer. These are interesting and important suggestions. It would be nice to have some experimental data to support the hypothesis.”

We appreciate the comment by the reviewer suggesting that data supporting our hypothesis in nuclear and cytoplasmic localization of NURR1 may be the key to determining the mechanism by which NURR1 has its effects in breast cancer. However, the current study is limited by several practical matters in this regard. Firstly, it has been our observation that transient and stable overexpression of NURR1 in breast cancer cell lines is problematic due to a substantial
reduction in cell viability in the presence of exogenous NURR1. Secondly, the mechanisms by which the subcellular localization and transcriptional activity of NURR1 is regulated in breast cancer is poorly characterized, with several mechanisms being postulated in the literature for the regulation of NURR1 transcriptional activity. The elucidation of how NURR1 is regulated with regard to subcellular localization and transcriptional activity requires a high degree of involvement, and we feel that this meritorious pursuit would be best served in the context of a future self-contained study. We have edited the text to describe these limitations and to describe multiple possible mechanisms by which NURR1 may exhibit these dual effects in breast cancer as indicated below:

“Interestingly, mRNAs encoding splice variants of NURR1 have been characterized, and several of these presumed gene products have dominant negative effects with regard to NURR1 –dependent transcriptional activity[31][32]. As mentioned above, our early attempts to transiently overexpress NURR1 in breast cancer suggest that breast cancer cells are intolerant to NUR1 expression, resulted in rapid cell death (data not shown).”

Minor Essential Revisions

1. “Check for typo errors”

As described above, we have had a third party to review the manuscript for typographical and grammatical errors.

Associate Editor: Kay-Uwe Wagner

1. “…when I read this interesting manuscript, I have to agree with the reviewer who stated that it might be beneficial to over express the receptor in xenografted cells.”

We are in full agreement with the assessment that development of a NURR1 overexpression model would add to the significance of this manuscript. However, we have made several attempts to establish NURR1 overexpressing cell lines without success due to poor survival of NURR1 overexpressing breast cancer cells. As such, we are unable to establish a NURR1 overexpression xenograft model at this time. A substantially more nuanced approach will be necessary to establish NURR1 overexpression cell lines and the subsequent xenografts. We have included in the discussion a description of these observations. As such, we feel that the significant impact observed by the experimental silencing of NURR1 substantially makes the case that NURR1 expression is important in breast tumor progression.

“As mentioned above, our early attempts to transiently overexpress NURR1 in breast cancer suggest that breast cancer cells are intolerant to NUR1 expression, resulted in rapid cell death (data not shown).”

2. “The inclusion of normal (untransformed) tissues and cells as controls increases greatly the quality of the paper.”

In compliance with the associate editor’s suggestion, we have included Western blot comparing of NURR1 expression in protein lysates from normal breast, invasive breast carcinomas, and breast cancer cell lines which support our contention that loss of NURR1 is linked to oncogenic
transformation (Figure 1C). Additionally, the text has been revised to reflect these changes as indicated below:

“This specific silencing of NURR1 in transformed breast was confirmed in using Western immunoblots, where relative NURR1 expression was determined in lysates from normal breast epithelium, transformed breast, and established cell lines (MDA-MB-468 and MDA-MB-231). Loss of NURR1 expression is evident in cancerous breast as compared to normal breast, (Figure 1C).”