Author's response to reviews

Title: Acquired Genetic Alterations in Tumor Cells Dictate the Development of High-Risk Neuroblastoma and Clinical Outcomes

Authors:

Faizan H Khan (Faizan-Khan@ouhsc.edu)
Vijayabaskar Pandian (Vijayabaskar-Pandian@ouhsc.edu)
SatishKumar Ramraj (Satish-Ramraj@ouhsc.edu)
Mohan Mohan Natarajan (natarajan@uthscsa.edu)
Sheeja Aravindan (Sheeja-Aravindan@ouhsc.edu)
Terence S Herman (Terence-Herman@ouhsc.edu)
Natarajan Aravindan (naravind@ouhsc.edu)

Version: 3 Date: 11 May 2015

Author's response to reviews: see over
Author's Response to Reviews

Ms. Ref. No.: 1452812588152264
Title: Acquired Genetic Alterations in Tumor Cells Dictate the Development of High-Risk Neuroblastoma and Clinical Outcomes
Authors: Faizan H Khan, Vijayabaskar Pandian, Satishkumar Ramraj, Mohan Natarajan, Sheeja Aravindan, Terence S Herman and Natarajan Aravindan
BMC Cancer

Author's Response to Reviews:
We truly thank the reviewers’ for their insightful review and valuable suggestions. We have performed a series of experiments addressing each reviewers’ concerns and the new data are now included in the revised manuscript. Please see below for the point by point response to the reviewer’s critiques.

Editor’s comments:
A well written paper that combines classical cytogenetics with contemporaneous arrays and use the increased selection power of in vivo serial xenografts. We congratulate the authors. A material that should be verified on further cell lines and perhaps on xenografts of biopsies. Array CGH works could be also more corroborated; see the Castresana groups recent PLoS One. 2014; 9(11): e113105.
As advised, we performed additional experiments to verify the association of acquired alterations of identified candidates to that of tumor progression directly in a cohort of 25 human neuroblastoma patients. Tumor grade-dependent correlation of RALYL expression conformed our in vivo experimental observations. Also, as advised array outcomes data are extensively interpreted in terms of their influences in multifarious molecular signaling pathways and biological functions pertaining to tumor progression. The manuscript is carefully revised including the additional findings and interpretation of the data. Please see below for the point by point response to the reviewers’ critiques.

Reviewer 1
One cell line should not be enough for this kind of experiments. At least 3-5 cell lines should have been used.
The clones used to determine the acquired genetic alterations are derived from multifarious metastatic tumors. However, we agree with the reviewer that, since it is off single parental cell-line dependent animal-model, at times may result in false positives/negatives. To that end, additional experiments were performed to verify the association of such acquired alterations to the clinical prognosis. For this, association between such altered candidate (RALYL) and the pathological grade of the tumor were assessed in a cohort of 25 neuroblastoma patients. The neuroblastoma tumor grade-associated increase in the RALYL localization corroborated well with our in vivo experimental observations. The experimental methods and observed results are now included in the revised manuscript. Please see

Abstract: Page 2 Lines 71-73
Methods: Page 6 Lines 253-270
Results: Page 9 Lines 382-394
Discussion: Page 12 Lines 531-537
Figure: Figure 7
Figure Legends: Page 17 Lines 760-764

Array CGH analysis and further validation by RT-PCR has already been documented by several authors. The validation has not been done by western blot, and this should be required as well.
As advised, additional experiments are performed to validate the Array CGH outcomes with Immunoblotting. For this, the expression profile of RALYL as well as FOXP2 those showed gain in array CGH analysis and increased transcription with QPCR analysis were examined both in the clones of metastatic site derive aggressive cells (compared to the parental SH-SY5Y cell line) and in multifarious metastasized tumors in vivo (compared to the non-metastatic primary xenograft). Both the aggressive (MSDAC) clones and well as metastatic tumors exhibited heightened levels of RALYL and FOXP2 and substantially validates and CGH array outcomes. The revised manuscript now includes this additional data. Please see

Abstract: Page 2 Lines 66-68
Methods: Page 6 Lines 247-251
Reviewer 2
The manuscript provides excellent data on the genetic changes during the disease process. However, except listing some genes it is not able to give some insight how these gene changes may affect the aggressive behavior of neuroblastoma cells. This requires some more explanation on the genes affected and how these genes may function to promote metastasis and heterogeneity. These are two key elements of neuroblastoma which make the treatment difficult.

As advised, we included additional interpretation of the Array CGH, transcriptional and immunoblotting data particularly focusing on the relevance of the altered molecules in their involvement in tumor progression related- molecular signaling pathways, -biological functions and their defined role in tumor dissemination. For this we performed extensive ingenuity pathway analysis as well as published evidence data mining and the revised manuscript now includes an in-depth insight on their influence in tumor progression and metastasis. Please see

Results: Page 9 Lines 398-412
Discussion: Page 10 Lines 413-432
Figure: Figure S2
Figure Legends: Page 17 Lines 774-777
Page 18 Lines 778-780
Table: Table S1
Table Legend: Page 18 Lines 806-810