Reviewer's report

Title: TrkA is amplified in malignant melanoma patients and induces an anti-proliferative response in cell lines

Version: 5 Date: 11 May 2015

Reviewer: Ken Dutton-Regester

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Pasini and colleagues present a report on the amplification of 1q23.1 in melanoma through the use of array CGH and find an association with its amplification and an increase in primary tumor thickness. Within this region lies a candidate hotspot gene of NTRK1 (TrkA) which with following experimental analysis, was found to be mutated in 50% of primary melanomas. Following this, a series of experiments are presented to elucidate a role of TrkA in melanoma via the MAPK and AKT pathways and the authors suggest the data indicates a putative role in both neoplastic transformation and oncogene-induced senescence. This study has decent body of work and raises some interesting future experiments to further elucidate the role of TrkA in transformation and different genetic backgrounds, however, there are a couple of issues that need addressing and further clarification.

Minor Essential Revisions
- Line 108-110 Introduction: The authors state the “low resolution of the methodologies employed” in previous studies is a contributor to the lack of detection of novel candidate alterations. I would be cautious in using this statement as multiple previous studies have used higher resolution arrays in melanoma that what was used in this study. For example, Gast 2010 GCC; 49(8):733-45 and Dutton-Regester 2012 GCC; 51(5):452-61. I would suggest revising this statement to reflect more accurately the literature.
- Methods, line 154 “Genome profiling of clinical samples”. Although the details of the genome profiling method is referenced, it would be useful to know what the exact array was used without having to look it up in the original reference. Suggest including a quick sentence in the methods to include this info.
- Table S1, Age at diagnosis and Breslow thickness headings are accidently reversed and needs to be fixed
- Some of the cell morphology figures are not clear (at least in this version under review). In particular, Figure 3a/b and 4a/b is not high enough resolution to clearly see the morphology changes. This could be just a problem in the review article, but the authors should make sure it’s clear in the final version.
- Figure S5 and Figure 4, NFG Is labelled wrong (should be NGF) in NFG only treated boxes
- Line 519 correction: Gens > genes
- Line 627 correction, “might have be adopted” to “might have been adopted”

In the discussion there should be quick mention that there is the possibility that another gene within the minimal common region could be responsible for melanoma oncogenesis.

Major compulsory Revisions

I am curious to whether there was enough material on the samples used on the arrays to perform expression analysis (either using RT-PCR with RNA or Immunohistochemistry using the FFPE blocks) to see if there is a correlation between amplification and expression of the gene? Although the authors present an interesting hypothesis that TrkA expression may be controlled or selected against as the tumor progresses, there is also the possibility that TrkA is not the driving gene within the minimal common amplification region identified. It’s clear from the small number of cell lines used in this study that expression is not highly expressed. Have the authors considered using existing resources to look into this question in more detail, specifically the CCLE database for melanoma cell lines and probably more important, accessing the TCGA data? The CBioPortal can quickly present some interesting analyses with the TCGA data in respect to correlation of the levels of mRNA and copy number status of TrkA, and also in regards to clinical attributes including staging of the tumor. For example, total copy number (or mRNA expression) can be correlated with the staging of the primary tumor in the melanoma TCGA- one might even hypothesize based on the authors conclusions in this paper that expression of TrkA might be higher in earlier staged primary melanomas with TrkA amplification (as opposed to later stage primary melanomas with TrkA amplification). I feel this paper would be improved and more transparent if some of these data analyses would be included in the paper (most likely as supporting data). This would allow the reader to make more comprehensive conclusions about all the data available on TrkA.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests