Reviewer's report

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

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Reviewer: Gary Edward Gallick

The manuscript of Pasini et al. demonstrates complex roles of TrkA in melanoma. In an extension of the group’s previous CGH work, they demonstrate amplification of 1Q23.1 as a common locus in malignant melanoma from patients, corresponding with TrkA amplification. The frequency of this event (~50%) is a novel observation, and corresponds with tumor thickness and earlier onset of metastasis. Surprisingly, however, no statistical significance in survival was observed, a concern given the cell line work that follows. Although TrkA was found to be amplified in some cell lines, it was not expressed. An inducible TrkA was a sound strategy to determine the effects of its activity in vitro, but the growth inhibitory effect of NGF is in seeming contrast to the apparently metastasis-promoting affect of gene amplification in human specimens. The different relationship of Erk and Akt to growth regulation adds to the manuscript. Thus, there are several interesting, though difficult to explain, findings in the manuscript. There are some concerns, including:

Major compulsory revisions
(1) The survival data are very important and need to be shown in the “main” figures.
(2) Similarly, the activation of Erk and Akt pathways should be in the primary figures.
(3) The dose-dependence of NGF in eliciting Erk and Akt activation should be assessed.
(4) The cell line data are confusing, with TrkA amplified but not expressed. With this concern in mind, it would be very important to show expression (e.g. by IHC) in at least some of the human samples in which TrkA is amplified.
(5) The quality of the blots for p21 in Figure 5 is inadequate.

Minor essential revisions:
1. As the in vitro work is done with NGF, it would be important to assess NGF expression in the clinical samples.
2. The quality of Figures 3A and 3B are insufficient to assess the morphologic changes the authors describe.
3. It is important to quantify the effects of Erk and Akt inhibition in response to NGF, given the complex differential response of inhibiting these signaling
enzymes.
4. To this reviewer, the data do not support the possibility that TrkA signaling may be required for the onset of malignant melanoma, but not later, as it does not explain why humans with TrkA amplification develop metastases sooner. One would need to assess expression of TrkA earlier and later in the primary tumor to draw such a conclusion.
5. The discussion is long and overly speculative.
6. Some attention to grammar is required.

Discretionary revisions:

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**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