Reviewer's report

Title: MicroRNAs define distinct human neuroblastoma cell phenotypes and regulate their differentiation and tumorigenicity

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Reviewer: Luca Longo

Reviewer's report:

In their manuscript entitled “MicroRNAs define distinct human neuroblastoma cell phenotypes and regulate their differentiation and tumorigenicity” the authors sought for miRNAs which are differentially expressed in the three basic cell types observed in Neuroblastoma tumors and cell lines. Since these different cell types (i.e. N-cells, I-cells and S-cells) are associated with features such as tumor differentiation state and tumorigenicity, which ultimately influence patient survival, the authors propose these miRNAs for the development of novel therapeutic strategies in Neuroblastoma.

• Minor Essential Revisions

This is a well written manuscript and functional studies on candidate miRNAs have been well performed. However, in my opinion, the manuscript will greatly benefit if the results obtained in NB cell lines are confirmed in tumor tissues from neuroblastoma patients, for example by comparing candidate miRNA expression levels between groups of tumors with different differentiation features or stroma-rich vs stroma-poor neuroblastomas. The authors conclude that these miRNAs may be used as prognostic markers and for the development of novel therapeutic strategies. In this regard in vivo studies in an orthotopical mouse model of neuroblastoma would provide more evidence that expression/inhibition of these miRNAs may drive the differentiation status of murine neuroblastomas and hence the overall survival.

The main point that it is not so clear to me is the advantage in mixing miRNAs from the two N- and two I-type lines with those from the S-type lines to be used as a control. Moreover, I do not understand how this control mix allows identifying miRNAs associated with S-type cells as stated in the first paragraph of the Results (page 10, line 6).

I would suggest the authors to explain in the text which were the criteria for the selection of the five miRNAs from the first grouping (N/I vs Mix). Indeed, it is not clear why these miRNAs were selected among twenty, which were significantly differentially expressed between the two groups. For example, why mir29a was not further analyzed since it was the most statistical significant (Supplementary Table 1)? The same question may be asked for those miRNAs (mir124, mir375 and mir10b) chosen as for being higher expressed in N- compared to I-cells. For example, why did not the authors select mir383 and mir369-3p which showed the
highest ratio of N/I, or mir-7 that is reported to be modulated in neuronal differentiation (Chen H et al Biochem Biophys Res Commun. 2010 Apr 16;394(4):921-7)?

In the first sentence of the Background the authors report a 3-year survival rate of < 20% for high-risk patients, citing a 2003 reference. Although still unsatisfactory high-risk NB survival rate is a bit higher nowadays, as for example reported in the following manuscripts:


miRNA names written in the legend of figure 2 and those reported in figure 2 insets A, B, C, are mismatched.

mir-21 and mir-221 expression is reported to play an oncogenic role in other cancers, in contrast with what the authors have found in neuroblastoma lines. It would be of interest to provide functional experiments to demonstrate their involvement in non-neuronal differentiation, as those performed with mir-335.

In Figure 1 there is a spelling mistake for the name of the line SK-N-LP as it should read SK-N-LD as reported elsewhere in the paper.

In the set of experiments aimed to observe how candidate miRNAs are modulated after RA and BrdU induced cell differentiation, why is it not reported how miR-375 levels change after RA treatment (Figure 3B)?

• Discretionary Revisions

When describing the miRNA microarray in the Material and Methods section, it would add information stating how many miRNAs are contained in the arrays employed as well as how many were deleted prior to analysis.

Throughout the manuscript text the word “Neuroblastoma” is written both in extenso and abbreviated. This should be probably avoided.

Figure 4A is someway a bit misleading as at a first look the title of the insets (miR-335, HAND1 and JAG1) seems to refer to the second bar of the histograms.

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