Reviewer's report

Title: miRNAs associated with chemo-sensitivity in cell lines and in advanced bladder cancer

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Reviewer: Erik Wiemer

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The manuscript identifies differentially expressed miRNAs in bladder tumors related to survival length and chemotherapy response. A limited set of miRNAs are selected for further experiments using eight different bladder cancer cell lines. MiRNA modulation using antagomirs and mimics has a variable effect on cisplatin sensitivity. Striking is the dramatic effect some of miRNAs have on cell lines, this clearly makes it difficult to interpret the results and draw firm conclusion. There are several important concerns regarding the manuscript that need to be addressed:

Major compulsory Revisions

1. In addition to tables 2 and 3 it will be very illustrative to also present supervised hierarchical clusterings as this nicely indicates the different miRNA expression levels in the different tumor samples.

2. As the response of the various cell lines to cisplatin but – even more so - to the effects of miRNA modulation is very different the question arises which of the cell lines represent the bladder tumors best. Have the authors miRNA profiled the cell lines to determine which of the cell lines most closely resembles bladder tumors.

3. Result section, “cisplatin sensitivity of bladder cancer cell lines”: Apparently there was no association between cisplatin GI50 and miRNA expression in the cell lines. How was this conclusion reached i.e. which statistical analysis was used? Are the differences in GI50 values for the different cell lines statistically different? Can the authors explain the peculiar behaviour of the RT4 cell line in the experiment of Fig. 1?

4. Result section, “cisplatin sensitivity following miRNA knock down”: if the authors conclude that miR-138 downregulation increases cisplatin sensitivity in four cell lines one might also say that e.g. miR-296-5p downregulation makes certain cell lines more resistant to cisplatin. The latter may make sense if miRNA downregulation already has a dramatic effect on cell viability. The authors do not indicate whether the found differences are statistically significant, this is important see also point 5. In the legend to Figure 3 and 4 the explanation of the LF treatment is missing. Fig. 3B and 4B displays the relative cell viability expressed; are these viabilities normalised to the negative control molecule treated with cisplatin?

5. In the discussion section the authors speculate on which mRNA targets explain the effects they observe in their miRNA modulation experiments without
showing any experimental proof. If one observes a sensitization for cisplatin when a specific miRNA is knocked-down (e.g. in 253JBV treated with LNA-138) one expects the cells to become more resistant when the miRNA is overexpressed? In some cases this is indeed observed but in other cases not. Do the authors have a plausible explanation?

6. Please explain why according to Fig. S1 miR-138 is expressed in 253JBV whereas in Fig. 2 no expression of miR-138 is detected in this cell line.

Minor Essential Revisions

1. Abstract, results: In the text it is written that 19 miRNAs correlated with response to chemotherapy and 6 miRNAs with survival time. However in Table 2 and Table 3 18 miRNA and 9 miRNAs are listed, respectively.

2. Material & Methods: MicroFluridic should be Microfluidic cards

3. Table 1: Please indicate range for the age of the patients and for the overall survival figures.

4. Instead of the term knock-in I would rather use overexpression.

5. Legend Fig S1: Explain LF2000

6. Table S3: singelplex should be singleplex

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'