Reviewer's report

Title: Epigenetic silencing of miR-375 induces trastuzumab resistance in HER2-positive breast cancer by targeting IGF1R

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Reviewer: Ratna Vadlamudi

Reviewer's report:

In this study, authors examined the mechanisms that contribute to trastuzumab resistance of breast cancer cells. Using acquired resistance model cells, and whole genomic miRNA profiling, authors identified alternations in the levels of 9 miRs. In this study, they focused on the hypothesis that miR375 levels (one of the nine miRs identified in the screen) contribute to up regulation of IGF1R conferring trastuzumab resistance. Using biochemical, and reporter gene assays, authors demonstrated that miR375 indeed target IGF1R. Further, ectopic expression of miR-375 inhibited IGF1R expression, restored sensitivity to trastuzumab. Some evidence was also provided demonstrating genetic regulation of miR375. Utilizing 40 breast tumors samples, authors found the levels of miR-375 were inversely correlated IGF1R in clinical samples. Overall this is well designed study and results have implications for targeting trastuzumab resistance.

1. Page 10: This statement need to be corrected: “and had a significantly higher proliferation capacity in an MTT assay (p<0.05, Figure 1B)”. MTT assays presented do not measure cell proliferation rather they provide a relative measure of total viable cells.

2. Figure 3D. More information on the type of breast cancer tissues (n=40) used for this analysis (tumor type, status of HER2, whether tumors were treated with any HER2 targeting drugs, therapy response if available) need to be included in the methods, results and figure legend.

3. Fig 5 C, D. The location of amplified region in the miR-375 promoter need to be indicated as well as the rationale for testing this specific region in the promoter and key transcriptional elements etc. should be included.

4. Fig 5E. The quality of Western in Fig 5E is poor. Better quality images from new experiment should be included.

5. Authors presented no data on Invasion and metastasis. Therefore the statements in results and conclusion section refereeing to effect of miR-375 on invasion and metastasis should be deleted from text. Some examples: Page 4: Ectopic expression of miR-375 inhibited IGF1R expression, restored sensitivity to trastuzumab and suppressed tumor cell invasion. Page 12: Trastuzumab-resistant breast cancer cells exhibit survival and invasion advantages over parental cells; Page 14: we investigated whether miR-375
suppresses trastuzumab resistance and metastasis by targeting IGF1R.

**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