Author’s response to reviews

Title: Glutamate metabotropic receptor 4 (GRM4) inhibits cell proliferation, migration and invasion in breast cancer and is regulated by miR-328-3P and miR-370-3P

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Francesca Peruzzi (Reviewer 1): The authors performed in silico analysis and showed increased expression of GRM4 in luminal breast cancer. They further demonstrated that GRM4 expression inhibits proliferation, migration and invasion of MCF7 breast cancer cell line. In addition they found that miR-328-3p and miR-370-3p target GRM4 3'UTR and that overexpression of either miRNA into MB-231 cells overexpressing GRM4 result in increased migration and invasion. While this knowledge could be relevant the study could be improved by providing data supporting the biological relevance of miRNA/GRM4 expression, as detailed below.

Specific comments:
1) Please add description to Fig 1D and add Fig.1E to the description of the survival curve in the results section.
Response: We have added description to Figure 1D and Figure 1E as you required.

2) The authors convincingly show that GRM4 is a target for the two miRNAs. However, it is not clear whether those miRNAs could play a biological role in breast cancer. The data could be strengthened by showing the expression levels of miR-328-3p and miR-370-3p in MCF7, MDA-MB-
231 and in clinical samples, and by showing the effect of miRNA mimics and inhibitors on GRM4 expression in MCF7 and MB-231 cell lines, respectively.

Response: We have shown the expression levels of miR-328-3p and miR-370-3p in several breast cancer cells and in clinical samples in Figure 4. The effect of miRNA mimics and inhibitors on GRM4 expression in MCF7 and MB-231 cell lines has been shown in Figure 3F and Figure 3G.

Shu-Pin Huang (Reviewer 2): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

This is an interesting study regarding the important role of Glutamate metabotropic receptor 4 (GRM4) in breast cancer with solid functional studies.

I have some minor comments:
1: The rationale/background: the authors should mention more about why choose GRM4 for study (why not other GRM protein family) what the literature suggest?
Response: We have presented the reason why we choose GRM4 for this study. To our knowledge, the current studies about the relationship between GRM4 and breast cancer is very limited. Only one literature (Clin Cancer Res. 2005 May 1;11(9):3288-95) reported the expression of GRM4 in breast cancer. The idea of this study came from one of my friends who is studying GRM family members and find several important effects of GRM family members in malignancies. So we explored the mRNA expression of all GRM family members using TCGA database as the initial step of this study.

2: In the discussion part: since we know that breast cancer is adenocarcinoma, which have epithelium and stroma components, but the breast cancer cell line only represent the characteristics of epithelial part;

Could authors address the possible important role of GRM4 in epithelial-stroma interaction in breast cancer?
Response: Because the studies about the role of GRM4 in breast cancer, even in other malignant tumors, is very limited. To our knowledge, we couldn’t find the possible important role of GRM4 in epithelial-stroma interaction in breast cancer. It is a very nice suggestion which could guide our future research direction.