Reviewer 2 v.1

Comments to the Author

In this study the authors show that co-treatment with Gefitinib and miR-30a-5p mimic may suppress tumor growth in an acquired resistant model of EGFR-mutant lung adenocarcinoma (H1650GR). The study described in this manuscript provides some advancement over their previously published study (Ref. 16) indicating that their findings in H1975 and H460 lines (showing intrinsic resistance) are also relevant to H1650GR cell line and also to some extent demonstrates the efficacy of this combination in vivo. Additionally, they have also included luciferase assays in this study to demonstrate binding between miR-590-3p and PIK3R2 and PIK3CD. This study could be considered for publication in "Therapeutic Advances in Respiratory Diseases" after experiments are performed to address the comments/questions stated below.

Some of the conclusions drawn from the results are incorrectly worded. For example, the lack of dose-dependent decrease in pAKT despite decrease in pEGFR levels upon gefitinib treatment of H1650GR line indicates that AKT is activated despite inhibition of EGFR signaling in this line indicating that AKT is being activated by a different RTK, not EGFR. This does not indicate that "high concentration of gefitinib could effectively inhibit the phosphorylation of EGFR, but, not inhibit the downstream signal transduction in EGFR signaling pathway" as the authors suggest. This conclusion drawn by the authors could mislead the readers into misinterpreting that the EGFR signaling pathway was not effectively inhibited in H1650GR line.

Another instance of incorrect wording of interpretation of a result is listed below:

The authors say that "In all this four concentrations of gefitinib, there was expression detected for p-IGF1R, but not for p-MET (Fig. 1B), indicating that the status of IGF1R pathway was activated, and MET pathway was inactivated".

The MET pathway was not activated in this case. The interpretation "MET pathway was inactivated" would mislead the readers into believing that MET pathway was active without EGFR inhibitors and inactivated upon Gefitinib treatment.

This article should be edited to correct the grammar and incorrectly worded interpretations and results.

The concentration of gefitinib and AEW541 used in Fig. 2 should be indicated.

For fig. 3, the specific mutations introduced at the putative miR-30a-5p binding sites within PIK3R2 and PIK3CD must be indicated.

Also, is the expression of miR-30a-5p lower in H1650GR line compared to the parental line, H1650?

In addition to luciferase assays, the authors must also perform western blot analysis to show that miR-30a-5p downregulates the expression of PIK3R2 and PIK3CD in H1650R.

Also, can H1650GR be sensitized to Gefitinib by co-treatment with an AKT inhibitor? Also, does the co-blockade of IGF1R in H1650R lines (Fig. 2) that causes reduction in pAKT levels also result in increased killing of this cell line? This would indicate that this cell line is dependent on PI3K/AKT signaling for survival.

Also, the effect of the overexpression of miR-30a-5p alone on H1650 Xenografts must be shown to demonstrate if the combination has superior effect to miR-30-5p overexpression alone. Also, pEGFR/pERK staining should be included in Fig. 7 to demonstrate that Gefitinib effectively inhibited EGFR in vivo in their study.