This interesting paper by He et al. explores the role of miRNA 224 is esophageal squamous cell carcinoma (ESCC). The authors claim that miRNA 224 expression levels are higher in intraepithelial neoplasia and ESCC specimens than in matched normal tissues. In vitro they then show that miRNA 224 is associated with cell viability, apoptosis, invasion, migration and colony formation. Next they select two potential target genes of miRNA 224 (PHLPP1 and 2) and demonstrate that miRNA224 binds to target sequences in the 3’ UTR of these genes and downregulates activity in a luciferase reporter assay. They also demonstrate in vitro that overexpression of miRNA 224 results in downregulation of PHLPP1 and 2 proteins and increases AKT activity. Finally, the authors show that PHLPP1 and 2 protein levels are lower in ESCC tissues than in matched normal tissues and that protein levels negatively correlate with miRNA 224 expression in these tissues. The authors conclude that miRNA 224 acts as an oncogenic miRNA in ESCC by targeting PHLPP1 and 2 and increasing AKT activity.

Overall this paper presents interesting and novel data that should be of interest to ESCC or oncogenic miRNA’s. However there are several issues that need to be addressed by the authors.

Major Revisions.

1. The qRT-PCR methodology needs to be better described and/or corrected. Primer sequences for the RT primers and PCR primers for miRNA 224 and U6 need to be provided. Also, the methods state that expression was calculated as 2^(-Ct) but this is not what is shown in Figure 1A and 1C. Instead, the #Ct appears to be plotted or perhaps the -#Ct. This is confusing and harder to interpret than simply plotting the actual relative expression as described in the methods. This should be corrected.

2. Page 12, lines 10-15. This section is very confusing. ESCC cases were split based on miRNA 224 expression levels and this was used for calculations in Table 1 but is this necessary? Why not use expression as a continuous variable and compare the clinicopathologic features (as appears to be the case in Figures 1B and 1C)? Also, the difference between the two panels in figure 1C is unclear. What is the difference between TNM and pathologic stage?

3. Figure 6. Data should be shown from all 12 cases.

4. The conclusion that the oncogenic activity of miRNA 224 is a result of targeting
PHLPP1 and 2 and the subsequent AKT activation is likely but not proven. There is no direct proof provided that the invasion, migration etc. phenotypes are driven by PHLPP1 and 2 or by AKT activation. The authors should either attempt to make this connection or change their conclusions accordingly.

Minor revisions.

1. Is IEN the same as dysplasia? If so, which terminology is more commonly used for ESCC? This should be clarified and if appropriate, changed throughout the text.

2. Page 12, line 7. Please remove the term “significantly overexpressed”. As far as I can tell this is simply based on the arbitrary >2-fold increase and not a statistical test of significance.

3. Page 7, line 17 and 18. How many mir-224 mimics were used? It appears to be two but with completely different sequences. Please explain. It may be more helpful if all oligonucleotide sequences were provided in a table rather than in the text.

4. Page 15, line 12. This should read “increased AKT signaling).

5. In general, the figure legends could do a better job of describing the actual data presented with less emphasis on the methodology.

**Level of interest:** An article whose findings are important to those with closely related research interests

**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.