Reviewer’s report

Title: Deregulation of microRNAs Let-7a and miR-21 mediate aberrant STAT3 signaling during Human Papillomavirus-induced Cervical Carcinogenesis: Role of E6 Oncoprotein

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Reviewer: Bharat Aggarwal

Reviewer’s report:

The manuscript (BMC Cancer) entitled “Deregulation of microRNAs Let-7a and miR-21 mediate aberrant STAT3 signaling during Human Papillomavirus-induced Cervical Carcinogenesis: Role of E6 Oncoprotein” by Shishodia et al., describe the followings:

1. Aberrantly expressed and constitutively active STAT3 signaling plays a pivotal role in initiation, progression and activation of human papillomavirus oncogenes during cervical carcinogenesis.

2. However, the underlying mechanism(s) responsible for pleiotropic effects of STAT3 signaling is poorly understood.

3. In view of emerging regulatory role of microRNAs, let-7a and miR-21 that may interact with STAT3 signaling and its downstream effectors, present study was designed in HPV16-positive cervical cancer cells to assess the functional contribution of these miRs in STAT3 signaling in cervical cancer.

4. STAT3 silencing was done using STAT3-specific siRNA by transfecting SiHa cells.

5. Pharmacological intervention of STAT3 was done by using specific inhibitors like curcumin and stattic.

6. Specific targeting of miR-21 using miR-21 inhibitor and gain-of-function study of Let-7a mimic was also analyzed by transfection in SiHa cells.

7. In addition, abrogation of HPV oncoprotein E6 was also done by specific siRNA.

8. Functional silencing of STAT3 signaling in SiHa cells by STAT3-specific siRNA resulted in a dose-dependent decrease in cellular miR-21 level.

9. Pharmacological intervention of STAT3 using curcumin and stattic that abrogated STAT3 activation resulted in loss of cellular miR-21 pool.

10. Contrary to this, specific targeting of miR-21 using miR-21 inhibitor resulted in an increased level of PTEN, a negative regulator of STAT3.

11. Besides miR-21, gain-of-function study of Let-7a mimic reduced cellular STAT3 level.

12. Abrogation of HPV oncoprotein E6 by specific siRNA resulted in increased Let-7a and decreased expression of miR-21.
Based on these studies authors conclude the existence of a functional loop involving Let-7a, STAT3 and miR-21 which were found potentially regulated by viral oncoprotein E6. miR-21 and Let-7a along with STAT3 may prove useful targets for pharmacological intervention for management of cervical cancer. These are well done studies, data support the conclusions and the manuscript is well written. However, there are also some problems as outlined below:

Main comments:
1. Introduction is too long and unfocused.
2. How specific are curcumin and static as STAT3 inhibitors?.
3. Is there any evidence that miR-21 and Let-7a interact with STAT3?
4. Is it possible that decrease in STAT3 expression and miR-21 (Fig. 1C & 1D) due to loss of cell viability (Fig.1B). This needs to be ruled out.
5. Same logic as #4 may also apply to curcumin.
6. Figure 1A, 2A, 3A are redundant with panel B in the all figures shown.
7. Authors presented no evidence that they are dealing with active STAT3 as stated in discussion.
8. Discussion is too long and unfocused.