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

Version: 3
Date: 11 September 2014
Reviewer: Chitra Mandal

Reviewer's report:

Authors in this manuscript established a relation between miR-21 and Let-7a in HPVE6-mediated active STAT3 signaling in cervical cancer cells. Moreover, they demonstrated an existence of miRNA-mediated loop which is positively regulated by HPVE6 and responsible for aberrant STAT3 signaling during HPV-induced cervical carcinogenesis. Though it was well organized MS, it has some concerns as follows before publication:

Major comments:

Q1. Authors showed accumulation of PTEN after silencing of miR-21 in Figure 3 and claim that: “It is likely that STAT3-induced miR-21 forms an important part of positive feedback loop in cervical cancer cells that keeps various apoptosis-inducing death regulators including PTEN, under control and miR-21 inhibition alleviates PTEN suppression leading to abrogated STAT3 signaling.” This claim should be justified if they could demonstrate the status of STAT3 under this same experimental scenario. This experiment need to be included.

Q2. Authors demonstrated that silencing of HPV16 E6 results in a specific up-regulation of Let-7a and abrogation of miR-21 level (Figure 5). The author should demonstrate the status of STAT3 and PTEN under this condition?

Q3. Fold change expression generally calculated as ratio against the loading control. From Figure 3D, it is clear that miR-21 inhibitor treatment resulted in PTEN accumulation at 10 nM of dose but a further increase in inhibitor dose did not enhance PTEN level. But in Figure 3E, the bar graph showed dose-dependent increase in PTEN expression which is not justified with Figure 3D. The author should recalculate the fold change.

Minor points

Q1. The authors should use either h or hr throughout MS
Q2. The pSTAT3 (Y705) of Figure 2H is not displayed. Therefore, this figure can not be assessed by the reviewer. Need clear figure