SUMMARY

Present investigation attempted to correlate levels of 2 oncogenic microRNAs, miR-21 and let-7a, with constitutively active STAT3 and its clinical relevance during cervical carcinogenesis. Total microRNA pool from cervical cancer cell lines (SiHa, HeLa, CaSki and C33a) and cervical biopsies from normal (23), pre-cancer (23) and cancer lesions (56) were evaluated for levels of miR-21 and let-7a by RQ-PCR, and their association with expression and activation of STAT3, downstream targets of STAT3 (MMP-2 & MMP-9), and miR-21 (PTEN, TIMP-3) were examined. To correlate the clinical correlation study we performed in-vitro experiments. We performed silencing of STAT3 by siRNA or blocked its tyrosine (Y705) phosphorylation in SiHa cells by herbal derivative curcumin, or specific inhibitor of its tyrosine (705) form stattic.

The salient features of our study are as follow:

- Over-expression of miR-21 and down-regulation of Let-7a was observed in HPV16-infected cervical cancer cell lines and cervical pre-cancer and cancer cells.

- Higher miR-21 expression and low Let-7a levels were accompanied with aberrant expression and constitutive activation of host transcription factor STAT3 that increased with the increasing severity of the lesion.

- miR-21 over-expression showed well association with over-expression of MMP-2 and MMP-9 in cervical cancer cell lines and cervical pre-cancer and cancer tissue biopsies.

- miR-21 expression was negatively correlated with the expression of PTEN and TIMP-3 in cervical pre-cancer and cancer lesions.

- Elevated level of miR-21 and low levels of Let-7a also correlates with high expression of HPV16 oncogenes E6 in cervical cancer cell lines and cervical cancer cases.

- Down-regulation of STAT3 by specific siRNA inhibited STAT3 expression along with decreased miR-21 expression in cervical cancer cells.
In addition, targeting STAT3 by curcumin and statric molecule showed decreased STAT3 activation which resulted in reduced miR-21 expression levels in SiHa cells.

Targeting of miR-21 by specific microRNA hairpin inhibitor that targets endogenous miR-21 resulted in the increase of PTEN protein pool.

Intracellular delivery of let-7a mimic that functions similar as mature miRNA in cervical cancer cells reduced the cellular STAT3 transcripts.

Specific silencing of viral oncoprotein E6 by E6 siRNA in these cells resulted in the accumulation of Let-7a and reduced the expression of miR-21 in cervical cancer cells.

Our results demonstrate, for the first time, an existence of a functional signaling pathway involving Let-7a, STAT3 and miR-21 which was found regulated by human papillomavirus oncoprotein E6.