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Thesis Topic: **Role of Signal Transducers and Activators of Transcription 3 (STAT3) in Human Papillomavirus 16(HPV16) induced cervical carcinogenesis**

ABSTRACT

The causal relationship between high risk-human papillomaviruses (HR-HPV) infection and cervical cancer has become evident from epidemiological and experimental studies. The oncogenic potential of the papillomaviruses can be attributed to expression and activity of E6 & E7 whose gene products functionally p53 and the retinoblastoma (Rb) proteins, respectively. Expression of HR-HPV E6 and E7 of HPVs is highly regulated by discrete enhancer elements located on <1kb length upstream regulatory region, LCR (Long Control Region) that controls activity of P97 promoter and drive transcription from these viral oncogenes. Recent study demonstrated presence of potential binding sites in HPV16 LCR for host transcription factor STAT3 which plays a pivotal role in epithelial carcinogenesis. However, the expression, activation, functional relevance and mechanism of action of STAT3 with respect to transcription of viral oncogenes, viral load and physical status of HPV genome in host cell have remained an unexplored area. In view of the above, in the present investigation status of STAT3 expression and activity was examined in cervical pre-cancer and cancer lesions and correlated with viral load and physical state of the virus and the association between active STAT3 levels with expression of viral oncogenes E6 & E7 in HPV16 infected lesions. In addition various approaches like STAT3 specific siRNA, AG490 as well as curcumin, inhibitors of STAT3 activation have been utilized to determine the role of STAT3 in HPV16 mediated cervical carcinogenesis. The study was performed on biopsies collected for diagnostic purposes from prospectively enrolled patients with different stages of cervical pre-cancer [LSIL (n = 50); HSIL (n = 70)] and cancer lesions (n = 100) alongwith normal control cervical tissues (n= 32) and established HPV16 positive and negative cervical cancer cell lines, SiHa, CaSki, and C33a. HPV infection was determined by using HPV L1 consensus PCR and genotyped using type-specific PCR as well as PGMY-PCR followed by reverse line blot. Cervical tissues and cell lines were analyzed for expression of STAT3 and phospho-STAT3, by immunoblotting and IHC.

Exclusive high prevalence of HPV16 was observed in cervical pre-cancer and cancer cases. Low levels of STAT3 in a subset of low grade pre-cancer lesions were observed, whereas a moderate STAT3 positivity was observed in high grade pre-cancer lesions. In contrast, in majority of

cancer cases STAT3 expression level was found to be either high or moderate. On the other hand, HPV16 positive cell lines, SiHa and CaSki, expressed higher levels of STAT3 compared to HPV negative, C33a cells. Analysis of the viral load in HPV16 positive pre-cancer and cancer lesions revealed an increased in the HPV16 viral load with increase in the disease severity from LSIL to HSIL and was the highest among cancer cases. Analysis of viral load with respect to expression of pSTAT3 (Y705) demonstrated significantly higher viral load in samples that expressed moderate or strong pSTAT3 expression in LSIL, HSIL and SCC cases. Integration analysis of HPV16 revealed LSIL cases primarily harbored episomal form of HPV16 whereas episomal HPV16 was also detected in about one half of the HSILs, but a significant number of cases possessed HPV16 DNA in either mixed or integrated state. Interestingly, majority of SCC cases were having either completely integrated (52%) or mixed form (41%) of HPV16 genome. In LSIL cases with episomal HPV16, STAT3 activation was found to be weak. On the other hand, a significant number of cases in HSIL and SCC category harboring either mixed or integrated form of HPV16 genome showed elevated expression level of active pSTAT3.

Cervical lesions with a moderate or high level of active pSTAT3 demonstrated correspondingly high levels of HPV16 E6 and E7 expression whereas expression of p53 and pRb proteins was undetectable in majority of these lesions. In contrast, cancer lesions with low pSTAT3 expressed low levels of E6 and E7 and correspondingly high p53 and pRB expression. The pSTAT3 expression was found to be significantly associated positively with HPV16 E6 & E7 expression and negatively with cellular p53 & pRb levels in cervical cancer lesions. RNA interference of STAT3 in HPV16 positive SiHa cells by STAT3 siRNA resulted in a specific decline in expression level of STAT3 and was accompanied with a concomitant reduction in pSTAT3 levels. Interestingly, accumulation of p53 and pRb was observed which increased proportionately with increasing dose of STAT3 siRNA with a concomitant loss of HPV16 E6 and E7 oncogene expression.

Interestingly, curcumin and AG490-mediated suppression of STAT3 activity was found inversely correlated with accumulation of cellular p53 and Rb levels. Expression analysis of HPV16 E6 and E7 oncoproteins of cellular proteins isolated form curcumin and AG490 cells also revealed loss of HPV16 E6 and E7 expression similar to one observed in STAT3-siRNA treated cells. Analysis of curcumin and AG490-treated cells revealed induction of apoptosis in cells with inhibited STAT3.

Overall, the leads obtained from the present study strongly support essential requirement of over-expressed and active STAT3 in cervical carcinogenesis for expression of viral oncogenes, E6 and E7, and silencing of p53 and pRB-mediated mechanisms of cell growth arrest. Taken together, STAT3 potentially represent a class of transcription factor that functionally interact to facilitate both, viral persistence and expression of viral oncogenes, in the infected host cell and thus play a central role in progression of the cervical neoplastic lesions. Therefore, the study provides an important background for developing novel STAT3-based approaches for therapeutic interventions against HPV infection and control of cervical cancer.