Discussion
**Discussion**

Signal transduction in cell involves the mechanism(s) by which transfer of biological information occurs leading to change in cell functioning. Over the last few decades considerable progress has been made in our understanding of signal transduction pathways. A number of signaling molecules which transmit extracellular signals to bring about changes in gene expression have been identified. The alterations in gene expression are controlled by transcription factors which are often located and/or translocated in the nucleus in order to activate gene transcription. According to Global Human Genome Analysis most of the proteins are encoded by transcription factors which are mostly over represented in cancer after kinases (Futreal et al., 2004; Ren et al., 2000; Tupler et al., 2001). Of several transcription factors, Signal Transducer and Activator of Transcription (STAT) have emerged as an important class of transcription factors that play a central role in converting extracellular signals into changes in expression of specific downstream target genes and, thereby, regulating complex biological processes, which they either stimulate or inhibit during development of many cancers (Darnell, 2002; Thierry, 2009; Yu & Jove, 2004; Yu et al., 2007). However, these transcription factors do not function in isolation but form regulatory networks in which several factors interact with them at a DNA binding domain also called transactivation domain that mediates the interaction between host and environment including viruses (Sibbet & Campo, 1990).

A number of oncogenic signalling pathways converge on a limited set of nuclear transcription factors (Cantley, 2002; Darnell, 2002; Hahn & Weinberg, 2002). These transcription factors termed as the final ‘switches’ activate the gene-expression patterns and ultimately lead to malignancy. Because of these properties, transcription factors are the ultimate workhorses of the cell and are targeted in anticancer treatments (Darnell, 2002). Targeting a single
transcription factor can block the effects of multiple upstream genetic aberrations that cause its persistent activation. However, transcription factors need to fulfill four main criteria to become an ideal target for cancer therapy. Firstly, these must be overactive in a large percentage of cells in different tumour types. Secondly, this activity should determine gene-expression patterns that not only promote cancer cell survival and proliferation, but also promote other malignant properties, such as tumour angiogenesis and immune evasion. Thirdly, good therapeutic targets must also be susceptible to specific inhibition by small-molecule drugs, and lastly tumour cells should be more dependent on the activity of the target transcription factor than normal cells. On the basis of these criteria, STAT (signal transducer and activator of transcription) proteins especially STAT-3 and, to a large extent, STAT-5 have emerged as promising molecular targets for cancer therapy (Yu and Jove, 2004).

Cancer of the uterine cervix is the second most common cancer among women worldwide with more than 80% cases occurring in developing countries (Das et al., 2008). In India, cervical cancer is a leading cancer among women with annual incidence of about 130,000 cases and 70-75,000 deaths (Das et al., 2008; Parkin et al., 2005). Thus India shares about one fourth of the global cervical cancer burden. A large number of risk factors are known to contribute to high incidence of this disease, but most important of them are early age of marriage (<18 years), multiple sexual partners, multiple pregnancies, poor genital hygiene, smoking, use of oral contraceptives, religion, ethnicity (Das et al., 2008). The most important factor however, has been considered to be the infection of human papillomaviruses (HPVs).

Since the HPV does not have its own transcriptional machinery, the expression of its two transforming oncogenes, E6 and E7 depends primarily on availability of host cell transcription factors (Abdel-Latif et al., 2009; Angel & Karin, 1991; Sen & Baltimore, 2006; Thierry,
2009; Timmons et al., 2002), However, currently there is no study that defines the role of STATs in HPV mediated cervical carcinogenesis.

**Role of STAT-3 in cervical cancer: Impact of HPV infection**

Signal Transducers and Activator of Transcription (STAT), particularly STAT-3 and STAT-5 play a role in the cell transformation and tumour progression by stimulating cell growth, promoting tumour angiogenesis, mediating immune evasion and conferring resistance to apoptosis induced by chemotherapeutic agents (Niu et al., 2002; Wang et al., 2004). However, whether STAT-3 plays any role in HPV mediated cervical carcinogenesis is not known. In the present study an attempt have been made to demonstrate the way STAT-3 gets deregulated in cervical cancer and how HPV infection could influence STAT-3 in the process as most of the cervical cancer tissues irrespective of their clinical and histopathological grade showed a high expression of STAT-3 as compared to normal controls. This is in agreement with recent study which demonstrated elevated levels of STAT-3 in endometrial and cervical cancer (Chen et al., 2007) and in ovarian cancer (Huang et al., 2000). Therefore elevated expression of STAT-3 seems to be one of common molecular mechanisms to promote oncogenesis in ovarian (Huang et al., 2000), endometrial (Chen et al., 2007) and cervical (Chen et al., 2007; Sobti et al., 2009) cancers. A significant correlation of STAT-3 expression with the stage of the disease was seen and it increased with the severity of the cervical lesion. Interestingly, STAT-3 has been found to show elevated expression in early stages of cervical cancer. This suggests that inhibition of STAT-3 expression in early stages of cervical cancer might prevent further progression of cervical cancer.

The present study also investigated the activation of STAT-3 in cervical carcinomas with an idea to better understand the mechanism of progression of cervical cancer involving the activation of multiple oncogenic pathways including the constitutive activation of STAT-3.
Discussion

The elevated levels of STAT-3 phosphorylation were detected in 47% of the total cervical cancer tumour biopsies as compared to normal controls, where it was almost negligible. This is in agreement with a recent study which demonstrated elevated levels of STAT-3 in endometrial and cervical cancer in American population (Chen et al., 2007) and in ovarian cancer (Huang et al., 2000).

STAT-3 activity is also induced by viral and bacterial infections. Previous studies showed interaction of STAT-3 with viruses and bacteria in the progression of various cancers, the activation of STAT-3 by the Hepatitis C virus core protein leads to cellular transformation (Yoshida et al., 2002) and another study demonstrates STAT-3 as a downstream target of Helicobacter pylori in gastric cancer (Tebbutt et al., 2002).

As HPV is an essential etiological factor in the cervical cancer progression, the present results were stratified on the basis of expression levels of STAT-3 in relation to absence or presence of HPV infection. An elevated level of STAT-3 expression was observed in HPV16/18 positive cases as compared to HPV 16/18 negative malignant samples. Therefore, these results indicated a potentially interactive effect between HPV and transcriptional activation of STAT-3 gene in cervical carcinogenesis. It has been shown that in non-malignant lesions, the HPV DNA persists as an extra chromosomal episome in the nucleus of the infected cells (Wentzensen et al., 2002). HPV DNA integration occurs in malignant lesions which increase the stability of mRNAs encoding the viral E6 and E7 oncogenes leading to elevation in viral expression (Jeon et al., 1995). This elevation in E6 and E7 expression affects the cellular signaling and particular cellular transcriptional factors (zur Hausen, 2000). HPV DNA integration also causes alterations in the integrity and expression of cellular genes such as MYC and APM-1 (Couturier et al., 1991; Reuter et al., 1998) and transcription factor AP-1 in esophageal (Hussain, 2009) and cervical cancer (Prusty & Das, 2005).
Thus, elevated expression of STAT-3 appears to contribute to more aggressiveness of the disease, as majority of these patients were infected with HPV and in poorly differentiated state and advanced stage of cervical cancer.

The present study also showed that STAT-3 phosphorylation is elevated in clinical human cervical cancer samples. Since STAT-3 was found to be activated in cervical cancer tissues and STAT pathway has been shown to participate in oncogenesis (Bromberg et al, 1999; Bowman et al, 2000), it appears to be one of the oncogenic pathways activated in human cervical cancers (Chen et al., 2007).

The activation of STAT-3 was seen to be significantly associated with HPV infection in cervical cancer. The present study revealed a significantly elevated level of activated STAT-3 (pSTAT-3) in cervical cancer cases infected with HPV as compared to HPV negative ones. Therefore, it is speculated that HPV plays a role in activation of STAT-3 in cervical cancer. Though this is the first study to report a possible association of pSTAT-3 and HPV infection in cervical cancer, many previous studies have reported association STAT-3 activation with other viruses (Kong et al., 2003; Waris et al., 2005; Yoshida et al., 2002). In another recent study in gastric cancer, H. pylori bacteria was found to activate the STAT-3 signaling pathway both in vitro and in vivo, providing a potential mechanism by which chronic H. pylori infection promotes the development of gastric cancer (Bronte-Tinkew et al., 2009). Though the mechanism behind the activation of STAT-3 by HPV needs to be explored, a previous study demonstrated that STAT-3 was activated in response to oxidative stress induced by HCV virus (Waris et al., 2005). The alteration of STAT-3 redox status could directly alter its conformation in such a way that its interaction with cytosolic proteins responsible for nuclear targeting is initiated. The other possible explanation is the ability of oxidants to act as inhibitors of tyrosine phosphatases, thereby inducing STAT-3 nuclear
translocation by enhanced tyrosine phosphorylation (Waris et al., 2005).

Since STAT-3 expresses differentially in non-neoplastic and neoplastic tissues contributing both in early events of tumourigenesis and tumour progression, elevated STAT-3 expression and transactivation appears to be generic carcinogenesis associated event.

**Role of STAT-5 in cervical cancer**

Another important member of the STAT family, STAT-5 gene has been demonstrated to play a crucial role during carcinogenesis since it is involved in promoting cell-cycle progression, cellular transformation and in preventing apoptosis (Calo et al., 2003). The overexpression of STAT-5 has been reported in many tumours including squamous cell carcinoma of the head and neck, lung, prostate and breast cancers (Haura et al., 2005; Sordella et al., 2004; Xi et al., 2003a; Xi et al., 2003b). Different isoforms of STAT-5 have distinct role in cancer. STAT-5a has been found to play an important role in the progression of breast cancer (Cotarla et al., 2004) while STAT-5b contributes to carcinogenesis in head and neck (Leong et al., 2002; Xi et al., 2003b). However, the key functional role of each STAT-5 isoform is not fully understood.

In the present study, STAT-5 protein was found to be upregulated in cervical cancer. Further investigation into the distinct role by STAT-5 isoforms revealed the deregulation of STAT-5 isoforms for the first time in cervical cancer. An elevated expression pattern of STAT-5b in most of the cervical cancer cases was observed. Overexpression of STAT-5b was also associated with degree of severity of cervical lesion. These findings are in agreement with a previous studies which showed expression of STAT-5b was significantly associated with advanced tumour stages and aggressive behaviour of cancer cells (Lee et al., 2006b). In contrast, normal tissues showed either low or undetectable expression of STAT-5b. (Ahonen et al., 2003) observed similar expression pattern of STAT-5b in prostate
cancer. The oncogenic potential of STAT-5b can be explained by the fact that blocking of STAT-5b causes downregulation of target genes that control several key events such as cell cycle arrest, apoptosis etc. due to deregulation of cyclin D1 and Bcl-X<sub>L</sub> family of proteins (Xi <em>et al.</em>, 2003b).

Most interestingly, in a reverse correlation with the upregulated STAT-5b expression, the other isoform STAT-5a revealed a gradual downregulation with increasing disease severity and became almost nil in invasive cancer. STAT-5a was downregulated in 81% of the cervical tumour tissues. In agreement with the above results, a study on hepatocellular carcinoma showed that activation of STAT-5a was absent in HCC clinical samples (Lee <em>et al.</em>, 2006b). Another study provides evidence that STAT-5a can act as a tumour suppressor by targeting suppression of key oncogene (Zhang <em>et al.</em>, 2007). The possible mechanism behind the downregulation of STAT-5a is not fully understood. (Zhang <em>et al.</em>, 2007) showed epigenetic gene silencing of STAT-5a by DNA methylation. Since it is well established that DNA promoter methylation is a common event of gene silencing in a variety of tumours (Baylin & Herman, 2000; Neyaz <em>et al.</em>, 2008; Salam <em>et al.</em>, 2009) therefore, it is quite likely possible that STAT-5a expression may be regulated by promoter methylation.

A variety of cytokines and growth factors including EGFR and prolactin have been shown to stimulate STAT-5 activation (Kloth <em>et al.</em>, 2002; Li <em>et al.</em>, 2004). Apart from physiological regulators activation of STATs is induced by viral and bacterial infections (Chen <em>et al.</em>, 2001; Hussain <em>et al.</em>, 2009; Lee <em>et al.</em>, 2006b; Sobti <em>et al.</em>, 2009; Yoshida <em>et al.</em>, 2002). Previous study by Lee <em>et al.</em> have shown that activation of STAT-5b was mediated by Hepatitis B virus infection in hepatocellular carcinogenesis (Lee <em>et al.</em>, 2006b). It has also been reported that STATs are a driving force for EBV gene expression in nasopharyngeal carcinoma and Hodgkin’s disease tissue samples and deregulation of JAK-STAT pathway may play a crucial role in EBV-associated...
tumourigenesis (Chen et al., 2001). Another study also demonstrates that cellular transformation was induced through activation of STAT-3 by the Hepatitis C virus core protein in hepatocellular carcinoma (Yoshida et al., 2002). Moreover, our recent study showed a strong association between HPV infection and STAT-3 overexpression in cervical cancer (Sobti et al., 2009).

In the present study almost 89% of the HPV infected cervical cancer cases showed a strong expression of STAT-3 and that of STAT-5b in 80% of HPV positive cases. Modification of the host cell transcriptional regulation is one of the major ways to regulate HPV production and maturation (Thierry, 2009). Since HPV does not have its own transcriptional machinery it primarily depends on the host cell machinery. HPV DNA integration occurs in malignant lesions which increase the stability of mRNAs encoding the viral E6 and E7 oncogenes leading to elevation in viral oncogene expression (Jeon & Lambert, 1995). Persistent viral infection in combination with strong, constitutive expression of E6 and E7 viral oncogenes is a necessary step for malignant transformation because these proteins interact with host cell machinery such as p53 and pRB proteins leading to their degradation and deregulation of the cell cycle (Boyer et al., 1996; Scheffner et al., 1990). This elevation in E6 and E7 expression affects cellular signaling and particular cellular transcriptional factors (Hussain et al., 2009; Prathapam et al., 2001; Prusty & Das, 2005; zur Hausen, 2000). This interaction between the HPV and host cell is supported by the well established fact that HPV viral gene expression is generally regulated by several viral and host-cell transcription factors, which bind to the Upstream regulatory region (URR) of the HPV genome. These factors include nuclear factor-1 (NF-1), activator protein-1 (AP-1), progesterone receptor, SP-1, Oct-1, YY-1, glucocorticoid receptor etc (Munoz et al., 2003). In the present study almost 90% of the cervical cancer cases were infected with HPV and interestingly all these cases harbored the most prevalent high-risk HPV type 16. Taken together these observations raised the possibility
that HPV infection may play a significant role in STAT-3 and STAT-5b upregulation in cervical cancer.

Another factor that could significantly contribute to upregulation of STAT-3 and STAT-5 is cigarette smoking which is a potent risk factor in cervical carcinogenesis (Castellsague & Munoz, 2003; Giuliano et al., 2002). Interestingly, majority of the cervical cancer cases reflected in the present study that showed STAT-3 and STAT-5 upregulation were active smokers. Previous studies have also demonstrated that smoking which contains a carcinogenic compounds such as nitrosoamines can lead to activation of host cellular transcription factors such as AP-1 and NF-kB (Anto et al., 2002). Also FOXM1 gene upregulation in human squamous cell carcinoma and Bcl-xl gene expression in human breast epithelial cells was enhanced in response to cigarette smoke condensate (Connors et al., 2009; Gemenetzidis et al., 2009).

The mechanism of STAT-3 and STAT-5 upregulation in human cancers is not clear and may probably depend on the specific cell type and activating stimuli in the tumour microenvironment. In hepatocellular carcinoma STATs particularly STAT-3 and STAT-5b were activated by Hepatitis B virus infection, on the other hand, in prostate cancer STAT-5 was shown to be activated by prolactin. In the present study HPV infection is implicated to play a crucial role in STAT-3 and STAT-5 upregulation in cervical cancer.

Role of SOCS-1 in cervical cancer: Impact of HPV infection

It has been reported recently that activation of JAK/STAT pathway plays a crucial role in the development of human cancer (Yu & Jove, 2004; Yu et al., 2007). It has also been observed that STAT-3 is constitutively activated in breast cancer, prostate cancer and leukemia (Li & Shaw, 2002; Lin et al., 2000; Schuringa et al., 2000). We have also for the first time demonstrated overexpression of STAT-3 in HPV mediated cervical carcinogenesis (Sobti et al., 2009). These observations lead us to investigate the possible mechanisms which
lead to the STAT-3 over expression in cervical cancer. SOCS-1 is a potent inhibitor of the JAK/STAT pathway and represent as a tumour suppressor gene (Rottapel et al., 2002). It is because of this the expression of SOCS-1 gene in cervical cancer cases was analyzed and compared it with that of normal controls.

It has been demonstrated that when compared with expression of SOCS-1 in normal tissues, 58% (52/90) of the cervical tumour tissues expressed either undetectable or reduced SOCS-1 expression (>50% loss of expression). Previous studies have also demonstrated loss of SOCS-1 expression in various tumours (Komazaki et al., 2004; Oshimo et al., 2004; Sobti et al., 2006) but there is no such study that has described the role of SOCS-1 in HPV mediated cervical carcinogenesis.

The aberrant methylation of CpG islands on the promoter region is one of the mechanisms of transcriptional silencing, leading to inactivation of tumour suppressor gene activity (Baylin et al., 2001). Such modifications have been reported in tumour suppressor genes in many cancers including cervical cancer (Duenas-Gonzalez et al., 2005; Virmani et al., 2001). In order to investigate the functional cause of loss of SOCS-1 expression in cervical cancer cases, the promoter methylation of SOCS-1 gene was analyzed in cervical cancer cases and compared with normal controls.

An aberrant promoter methylation in 54% (49/90) of cervical cancer cases was observed and it significantly increased with the severity of cervical lesion as compared to normal controls where no methylation was observed. These results suggest that methylation of SOCS-1 gene plays a crucial role in the progression of cervical carcinogenesis. Previous studies have also shown that tumour suppressor genes displayed a significantly increased frequency of promoter methylation with severity of neoplasia (Flatley et al., 2009; Oshimo et al., 2004; Tanemura et al., 2009).
The comparison of cases showing alterations in SOCS-1 expression with promoter methylation revealed that 90% (47/52) of the cases which showed either undetectable or significantly reduced SOCS-1 expression (>50% loss). On the contrary, normal controls in which no methylation of SOCS-1 promoter was detected demonstrated elevated levels of SOCS-1 expression. Thus the present study suggested an evidence of SOCS-1 promoter methylation may be one of the mechanism(s) in the silencing of this gene. These observations are in agreement with previous studies that have reported methylation mediated gene silencing of SOCS-1 with gene promoter hypermethylation ranging from 32% to 65% in other cancers such as hepatocellular carcinoma, multiple myeloma and pancreatic cancer with resultant activation of STAT-3 (Buslei et al., 2006; Fukushima et al., 2003; Galm et al., 2003; Hatirnaz et al., 2007; Tischoff et al., 2007; Yoshikawa et al., 2001) (Table D1).

It is quite likely that DNA methylation may directly interfere with basal transcriptional machinery by altering its secondary structure, especially the major groove conformation. Based on the evidence on a global examination of CpG methylation in a large number of tumours differential pattern depending upon tumour type and site was seen (Costello et al., 2000). The current study has demonstrated a direct association between lack of protein expression at the cell surface and a decrease in mRNA levels of SOCS-1 gene in cervical cancer cases, suggesting thereby transcriptional downregulation of SOCS-1 during cervical cancer progression due to promoter methylation. A significant correlation between promoter methylation and loss of SOCS-1 expression with increasing stage and histological grade of the disease has also been observed. Moreover, when the current findings on the role of SOCS-1 in cervical cancer were analyzed with respect to STAT-3 overexpression in the same subset of samples as studied earlier (Sobti et al., 2009), most of the cases that showed loss of expression which may be due to SOCS-1 promoter hypermethylation had an elevated STAT-3 expression as
compared to normal controls. This implicated that methylation mediated gene silencing of SOCS-1 may act as a possible cause of overexpression of STAT-3 in cervical carcinogenesis. A study by Huang et al., 2008 has also shown that loss of SOCS-1 expression leads to elevated STAT-3 that played a crucial role in brain metastasis with enhanced tumour invasion and angiogenesis.

**Table D1 - Frequency of SOCS-1 methylation and loss of expression in various cancers.**

<table>
<thead>
<tr>
<th>Country</th>
<th>Source Reference</th>
<th>SOCS-1 alterations</th>
<th>Cancer type</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>(Yoshikawa et al., 2001)</td>
<td>65% Methylation</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td></td>
<td>(Tanemura et al., 2009)</td>
<td>increase in hypermethylation</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>(Zhou et al., 2007)</td>
<td>100% in cell lines</td>
<td>Glioblastoma</td>
</tr>
<tr>
<td></td>
<td>(Lee et al., 2006c)</td>
<td>40% Transcriptional silencing</td>
<td>HNSCC</td>
</tr>
<tr>
<td>USA</td>
<td>(Galm et al., 2003)</td>
<td>63% Inactivation by methylation</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>Japan</td>
<td>(Okochi et al., 2003)</td>
<td>60% 45% Methylation</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td></td>
<td>(Miyoshi et al., 2004)</td>
<td>Inactivation of translation, diminished expression of SOCS-1 mRNA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Oshimo et al., 2004)</td>
<td>44% Transcriptional silencing</td>
<td>Gastric cancer</td>
</tr>
<tr>
<td></td>
<td>(Fujitake et al., 2004)</td>
<td>8%</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>(Nagai et al., 2003)</td>
<td>47% Protein expression loss in 50%</td>
<td>Hepatoblastomas</td>
</tr>
<tr>
<td></td>
<td>(Nomoto et al., 2007)</td>
<td>55% N.D.</td>
<td>Hepatocellular cancer</td>
</tr>
<tr>
<td>Germany</td>
<td>(Tischhoff et al., 2007)</td>
<td>42% N.D.</td>
<td>Barrett’s adenocarcinoma</td>
</tr>
<tr>
<td></td>
<td>(Brakensick et al., 2005)</td>
<td>31% Transcriptional silencing</td>
<td>Myelodysplastic syndrome</td>
</tr>
<tr>
<td></td>
<td>(Buslei et al., 2006)</td>
<td>51% Reduced SOCS-1 expression</td>
<td>Pituitary adenomas</td>
</tr>
<tr>
<td>India</td>
<td>Present study</td>
<td>54% 58% cases showed transcriptional and translational silencing</td>
<td>Cervical cancer</td>
</tr>
</tbody>
</table>
When the impact of HPV infection on SOCS-1 promoter methylation and loss of SOCS-1 expression was analyzed, it was observed that almost 98% of the methylated cases were HPV positive and promoter methylation of SOCS-1 and loss of SOCS-1 expression was significantly co-related with HPV infection in advanced stage of the disease. These observations suggested that HPV infection plays a crucial role in conversion of normal cervical epithelium to cancerous lesions through the pre-cancerous stage, but the progression to advanced stage of disease was due to synergistic effect of HPV infection and suppression or overexpression of tumour suppressor genes and oncogenes during the process of carcinogenesis.

Based upon the above findings, it can be speculated that HPV infection may be playing a significant role in gene silencing of SOCS-1 through promoter methylation. The impact of other viruses like Hepatitis C virus (HCV) on SOCS-1 expression has been well explored in hepatocarcinogenesis. A previous study has revealed that frequency of SOCS-1 methylation to be more in HCV positive cases than its negative ones (Yang et al., 2003) and these findings were strengthened with the observation of another study which demonstrated that HCV core protein downregulates the expression of SOCS-1 gene and is an alternative method to hypermethylation leading to its silencing (Miyoshi et al., 2005). Moreover a study by Kamio et al (Kamio et al., 2004) demonstrated that when SOCS-1 was induced in HPV infected cervical cancer cell lines it interacted with the viral oncogene HPV E7 protein and induced ubiquitination and degradation of E7. As a result, the Rb protein levels increased and suppressed the proliferation of cervical cancer cell lines, infected with HPV. With this transforming potential of SOCS-1 and its significant role in regulation of E7 protein level it can be proposed that SOCS-1 can emerge as a new therapeutic tool for HPV mediated tumours.

Taken together, these observations suggested that SOCS-1 negatively regulates STAT proteins which in turn results in the deregulation of the JAK/STAT pathway, and is a common mechanism
in cervical cancer cases. Also, transcriptional inactivation of SOCS-1 gene, primarily due to DNA promoter hypermethylation and the synergism with HPV infection may be playing an important role in cervical carcinogenesis.

**Human pappilomavirus infection in cervical cancer**

Considering oncogenic role of HPVs which is an essential etiological factor in the development of cervical cancer (Das et al., 2008) and their interaction with transcription factors (de Wilde et al., 2008; Fontaine et al., 2000; Nees et al., 2001; Offord & Beard, 1990; Prusty et al., 2005), we analyzed the HPV infection status in cervical cancer. The present results demonstrated that large quanta of cervical cancer cases were infected with HPV infection accounting for about 90% of the total cases. Earlier studies in our lab on different subset of samples from the same region showed similar frequency of HPV infection (Katiyar et al., 2005). This is in concordance with previous epidemiological studies carried out world over that showed presence of HPV is as high as 99% of cervical tumours and established association of certain specific HPV types as the necessary cause of progression to cervical cancer and these are termed as high-risk HPV types (Chen et al., 2009; Clifford et al., 2003; De Vuyst et al., 2009; Munoz et al., 2006). In the present study 81% of the invasive cervical cancer cases were infected with HR-HPV type 16 as compared to 6% infected with HR-HPV type 18. This is in agreement with the previous studies which show that HPV 16/18 infection accounts for more than 80% of invasive cervical cancer (Clifford et al., 2003; Chen et al., 2009; De Vuyst et al., 2009)

Previous studies demonstrated that 85-90% cervical cancer cases in India were squamous cell carcinoma and HPV 16 is the most prevalent in them (Das et al., 1993). In the present study involves most of the cervical cancer cases had squamous cell carcinoma and harbored HPV type 16 as the most prevalent HPV type.

It is well established that HPV is important risk factor but not sufficient for cervical cancer progression. In addition, there are several
cofactors that may increase the progression of HPV infected cervical lesions to cancer. Cervical cancer persists as an international health problem and the global health impact of smoking on cervical cancer in cervical cancer and related mortality is substantial. Cotinine, a nicotine metabolite is present in measurable concentrations in the cervical mucus of active smokers (Poppe et al., 1995). It is also well documented that cigarette smoking plays a role in increasing the risk of developing cervical cancer (Wilkelstein et al., 1997; IARC, 2004). The present study demonstrates a significantly high number of smokers (89%) infected with HPV and most of the cases belonged to invasive cervical cancer. Passive smoking has also been shown to act as a risk factor for high risk HPV types infection (Coker et al., 1992; Sobti et al., 2006). In the present study almost 95% of the passive smokers were infected with HPV. These observations are in agreement with previous case-control and cross sectional studies which indicated that women married to smokers experience a higher risk of developing cervical neoplasia than those married to non-smokers (Tay & Tay, 2004). Patients with low parity status were found in normal controls while an increased parity status was found in higher stages of cancer and significantly correlated with HPV positivity. This is in agreement with the previous studies that reported high parity increases the risk of squamous-cell carcinoma of the cervix among HPV-positive women (Munoz et al., 2002). This outcome of the study further explains the decline in parity caused reduction in cervical cancer incidence recently as seen in most of the developed countries (Munoz et al., 2002).

Therefore, in the current study smoking, multiparity (i.e. high rate of child birth) and early age of marriage were found to be risk factors in the development of HPV mediated cervical carcinogenesis. The prevalence of cervical cancer in North Indian women with respect to their religion, education and economic status was analysed. Majority of cervical cancer cases were Hindus followed by Sikhs and almost nil were Muslims. Previous studies have shown male circumcision to be significantly associated with lower cervical cancer
incidence (Castellsague and Munoz, 2003). Also women with low economic background and education status appeared to suffer more with cervical cancer. This lack of awareness among Indian women especially in the rural areas is the cause behind the failure of the various cervical cancer screening programs.

A highly significant correlation was found between HPV infection and cervical carcinoma stages. Though HPV infection was more prevalent in advanced stage of cancer, the entry of HPV in cervical cancer appears to be at the pre cancerous stage as 75% of these showed HPV infection Therefore, HPV may be significantly influencing both disease initiation and disease progression.

An understanding of the molecular mechanisms of the signaling pathways that are involved in cervical cancer progression is important. Present investigations have provided further insight into the differential expression pattern of STAT-3 and STAT-5 in cervical cancer. The results have also indicated a potentially interactive effect between HPV and transcriptional activation/inactivation of STAT-3, STAT-5 and SOCS-1 in cervical carcinogenesis.

Based upon the above findings the following model which depicts the possible role of STATs in cervical carcinogenesis has been proposed.
Figure D1: Deregulation of STATs (STAT-3 and STAT-5) in HPV mediated cervical carcinogenesis.

The present study demonstrates the transcriptional upregulation of STAT-3 and STAT-5b and suggests that their possible synergism with HPV infection may play an important role in the process of cervical carcinogenesis. STAT-3 and STAT-5 participate in oncogenesis by regulating the genes for survival, proliferation and angiogenesis which include BCL-XL, cyclin D1 and VEGF. SOCS-1 is a negative regulator of STATs and has a tumour suppressor activity. Methylation-mediated gene silencing of SOCS-1, a negative regulator of STATs was observed in cervical cancer. (Figure D1)