INTRODUCTION

Cervical cancer is the third most common cancer and the fourth leading cause of cancer death in women worldwide. This female malignancy accounts for 9% (529,800) of newly diagnosed cancer cases and 8% (275,100) of the total cancer deaths among females in 2008. More than 85% of these cases and deaths occur in developing countries (Jemal et al. 2011). It is well established that HPV infection is the primary etiological agent of cervical cancer (Walboomers et al. 1999; Woodman et al. 2007; zur Hausen 2009) but there is no specific clinically available treatment for HPV infection (Stanley 2003). To date, over 200 different HPV types have been identified, and about 40 of these infect epithelial cells of the genital tract (Burd 2003; Shukla et al. 2009). On the basis of their association with disease types, papillomaviruses are classified into high-risk (HR) and low-risk (LR) types. High-risk HPV types are often associated with carcinoma of anogenital tract, whereas the low-risk HPV types are associated with low grade benign lesions, like skin, genital warts and condylomata acuminata and are rarely associated with malignancy. Among 15 high risk types, HPV16 and HPV18 are the most prevalent genotypes, as together they are responsible for more than 80% of global HPV-associated cancerous lesions.

Following discovery of HPV and its absolute involvement in initiation and progression of cervical cancer, several studies were conducted to assess the natural history of HPV infection during cervical carcinogenesis (Bosch et al. 1995; Bosch et al. 2002). These studies have established that cervical cancer progress through definitive pre-cancer stages that are marked by characteristic changes in epithelium of transformation zone of cervix and are broadly classified as low-grade squamous intraepithelial lesions (LSIL) or high-grade SILs (HSIL) (Woodman et al. 2007). Among 15 high-risk HPV types, HPV16 and HPV18 are the most prevalent genotypes, as they together are responsible for more than 80% of global HPV-associated cancerous lesions and has even higher prevalence in developing countries like India (Shukla et al. 2009). HR-HPVs encode oncoproteins E6 and E7 that play a vital role in tumorgenic transformation in cervical epithelial cells. The expression of viral oncogenes is tightly regulated by host transcription factor that directly or indirectly influence viral transcription by interacting with upstream regulatory region.
(URR) of viral genome that harbors cis-acting elements which control viral oncogene expression through regulation of viral promoter. Recent observations from our laboratory (Sobti et al. 2009; Shukla et al. 2010) and others (Chen et al. 2007; Takemoto et al. 2009) indicate involvement of an aberrantly expressed and constitutively active STAT3 in cervical carcinogenesis whose levels increase with progression of disease in HPV16-infected lesions. Recent report from our lab demonstrates a functional role of active STAT3 in cervical carcinogenesis. Aberrant activation of STAT3 has been strongly associated with carcinogenesis (Kim et al. 2007). STAT3 is activated primarily through phosphorylation at Tyr705 residue (Germain and Frank 2007). Although there are a number of positive and negative protein regulators that regulate expression and activity of STAT3, recent studies implicate involvement of different microRNA in controlling STAT’s downstream targets and STAT proteins levels and their activity as well (Kohanbash and Okada 2012). The mechanism by which STAT3 regulates and it is being regulated by microRNA in cervical lesions is yet an unexplored area.

Active STAT3 has multiple effects on cellular physiology and oncogenesis that are manifested through transcriptional switch of several promoter of genes associated with malignant transformation. Recent studies suggest STAT3 may exert its oncogenic role through microRNAs (Kohanbash and Okada 2012). Involvement of miRNA has been documented for almost all major cellular functions such as cell proliferation, cell differentiation, stress response, apoptosis and transcriptional regulation (Johnson et al. 2005; Chang et al. 2007; Garcia M et al. 2007). Multiple miRNAs have been found with altered expression in HPV-positive cervical cancer cells compared to normal cervical tissues (Martinez et al. 2008; Reshmi and Pillai 2008; Hu et al. 2010; Zheng and Wang 2011).

Since alterations of these biological processes are the hallmark of many cancers, dysregulation of miRNA biogenesis and function may lead to tumorigenesis. Accumulating evidence suggests the potential involvement of a small subset of miRNAs in initiation and progression in a wide range of human cancers that are broadly classified as oncomiR (Esquela-Kerscher and Slack 2006). Altered miRNA expression has now been reported in leukemia, lung cancer, and colon cancer (Calin et al. 2002; Michael et al. 2003; Calin et al. 2004). Alterations in cellular
miRNA patterns have been reported in cervical cancer tissue and cervical cancer cells (Lui et al. 2007; Martinez et al. 2008; Wang, X. et al. 2008).

Recent reports suggested that microRNA-21 (miR-21) functions as an oncomiR in human cancers (Chan et al. 2005; Iorio et al. 2005; Meng et al. 2006). The gene encoding oncogenic miR-21 is controlled by an upstream enhancer containing two STAT3 binding sites that are strictly conserved (Loffler et al. 2007). It was shown that when miR-21 was suppressed, cell growth inhibition and caspase-dependent apoptosis were observed in different types of cancer cells (Chan et al. 2005). The available literature suggests that among certain cancer-associated microRNA, miR-21 is specifically up-regulated in different cancers and thus referred to as oncomiR (Buscaglia and Li 2012). miR-21 has been found to control apoptosis, cell proliferation, and migration of cell lines in breast, colorectal, and other cancers (Meng et al. 2007b; Si et al. 2007; Asangani et al. 2008; Zhu et al. 2008). Interestingly, miR-21 is located in the fragile site FRA17B region, which is one of the HPV16 integration loci at 17q23.2 (Loffler et al. 2007). A recent study showed that miR-21 targets PTEN gene through a binding site on the 3’UTR in hepatocellular carcinoma (Meng et al. 2007a). PTEN has been shown to be a critical tumor suppressor gene that is commonly inactivated in GBM by deletion, mutation, or attenuated expression (Kato et al. 2000). However, the correlation of miR-21 expression with constitutively active STAT3 in cervical carcinogenesis is yet to be investigated.

Apart from miR-21, recent reports have revealed that Let-7 was found to be frequently down-regulated in many human cancers including tumors of colon, lung, and breast, (Takamizawa et al. 2004; Akao et al. 2006; Inamura et al. 2007; Sempere et al. 2007) and that the chromosomal region of human let-7 is frequently deleted in many cancers (Calin et al. 2004). It is important to note that STAT3 has been reported as direct cellular target of let-7a (Wang, Y. et al. 2010). Forced expression of Let-7 family members was found to suppress cancer cell growth both in vitro and in vivo (Esquela-Kerscher et al. 2008; Kumar et al. 2008; Trang et al. 2010; Trang et al. 2011). These studies suggested a potential role of Let-7a in up regulation of STAT3 that needs to be explore further. With a particular reference to
cervical carcinogenesis which is promoted by infection of HR-HPVs through expression of viral oncoproteins. There are recent studies that have illustrated the possible interactions between HPV and microRNAs. HPV infection in cancer cells was found to be able to negatively influence miR-34a levels through E6 inhibition of p53 (Wang, X. et al. 2009). E6/E7 was found to down regulate the tumor suppressor miR-218 (Martinez et al. 2008).

STAT3 proteins are also known to be involved in the modulation of activity for both promoters of MMPs and tissue inhibitors of MMPs (TIMPs) which are considered major oncogenic targets and may be responsible for malignant switching of tumors (Gatsios et al. 1996; Korzus et al. 1997; Catterall et al. 2001; Tsareva et al. 2007). TIMPs contain a putative consensus miR-21 binding site and reduced expression of TIMP3 in breast cancer has been associated with poor disease-free survival (Mylona et al. 2006). Moreover, a recent study showed that miR-21 targets PTEN gene through a binding site on the 3’-UTR in hepatocellular carcinoma (Meng et al. 2007b). A recent study showed that miR-21 targets PTEN gene through a binding site on the 3’-UTR in hepatocellular carcinoma (Meng et al. 2007a). PTEN has been shown to be a critical tumor suppressor gene that is commonly inactivated in GBM by deletion, mutation, or attenuated expression (Kato et al. 2000). PTEN is a negative regulator of STAT3 activation in HPV-infected cells (Sun and Steinberg 2002). Therefore, it is likely that let-7a and miR-21 might be working in tandem with STAT3 during cervical carcinogenesis; however, such possibilities have not been explored yet.

While the functional significance of the miR level changes is being addressed, available literature suggests that the miRNAs can also be classified as oncomiRor tumor suppressor miRs which underscore the potential significance of miRNAs in therapeutic and diagnostic applications (McManus 2003; Lee and Dutta 2006). However due to pleiotropy and redundancy of their targeting any particular transcript in the cell the likelihood of developing them as independent prognostic/diagnostic marker is relatively low. Therefore it is important to assess them in light of their ontogenetically relevant targets/regulators like STAT3.
In view of the above, in the present study we evaluated the expression levels of miR-21 and let-7a along with levels of STAT3 expression and its activation to examine their association in different cervical tissues derived from pre-cancer (LSIL and HSIL) and cancer lesions. In addition, to test the functional role of miR-21 and let-7a, we examined association of their levels with expression of downstream targets of STAT3 during cervical carcinogenesis. In the next part of the study, to understand the causes and consequences of aberrantly over-expressed and constitutively active STAT3 signaling in cervical cancer in relation to different miRNA we explored the role of miR-21 and Let-7a using different approaches to target expression/activity of STAT3, miR-21 and over-expression of Let-7a using specific mimics.

So keeping above background in mind, the present investigation was designed with objectives given in next section: