INTRODUCTION
Cancer is a collection of diseases all of which are characterized by uncontrolled cell growth. It is a disease of the genome. Early stages in cell transformation involve mutations in genes directly or indirectly controlling cell proliferation and differentiation. It has now been revealed that different forms of cancers share common molecular mechanism governing uncontrolled cellular proliferation, involving loss, mutation, or deregulation of genes that positively and negatively regulate cell proliferation, migration and differentiation (generally classified as proto-oncogenes and tumor suppressor gene).

Cancer is caused by abnormalities in the genetic material of the transformed cells (Vogelstein and Kinzler, 2004). These abnormalities may be due to the effects of carcinogens, such as tobacco smoke, radiation, chemicals, or infectious agents. Other cancer-promoting genetic abnormalities may randomly occur through errors in DNA replication, or are inherited, and thus present in all cells from birth. The heritability of cancer is usually affected by complex interactions between carcinogens and the host's genome. Nearly 12.7 million new cancer cases and 7.6 million cancer deaths occurred in 2008 worldwide (Globocan 2008 website: http://www.dep.iarc.fr/globocan/database.htm).

Cervical cancer is the third most common cancer in women, and the seventh overall, with an estimated 5,29,000 new cases in 2008. It is responsible for 2,74,000 deaths in 2008, about 88% of which occur in developing countries (Globocan 2008 Website: http://www.dep.iarc.fr/globocan/database.htm). India accounts for a quarter of the world burden of cervical cancer (Sankarnarayanan et al., 2003). Current estimates indicate that, in India, every year 1,34,420 women are diagnosed with cervical cancer and 72,825 die due to this disease. Cervical cancer ranks as the most frequent cancer among women in India (Globocan 2008, website: http://www.dep.iarc.fr/globocan/database.htm). An estimated 2,05,496 new cases and 1,19,097 deaths due to cervical cancer will occur in India by 2020.
contribute to 29% and 30% respectively of the global burden of cervical cancer cases and mortality (Ferlay et al., 2004).

The early stages of cervical cancer may be completely asymptomatic (Kumar et al., 2007). Vaginal bleeding, contact bleeding or a vaginal mass, which occurs rarely may indicate the presence of malignancy. Also, moderate pain during sexual intercourse and vaginal discharge are symptoms of cervical cancer. In advanced disease, metastases may be present in the abdomen, lungs or elsewhere. Symptoms of advanced cervical cancer may include: loss of appetite, weight loss, fatigue, pelvic pain, back pain, leg pain, single swollen leg and heavy bleeding from the vagina (Nanda et al., 2006) and bone fractures. It may present with vaginal bleeding but symptoms may be absent until the cancer is in its advanced stages (Kumar et al., 2007).

It is one of the most common diseases of women, which is characterized by infection of specific types of human papillomaviruses (HPVs) such as HPV type 16 and 18. Human papillomavirus (HPV) is considered as the primary causal agent in the development of cervical carcinoma. Walboomers et al. (1999) reported that HPV DNA was detected in 99.7% of 1000 evaluable cervical cancer biopsy specimens obtained from 22 countries. Among high risk strains, HPV16 and HPV18, are most closely associated with cervical carcinoma and are found in >50% and 20% of squamous cell carcinoma respectively (Walboomers et al., 1999). The discovery of the inactivation of tumor suppressor genes p53 and RB by E6 and E7 oncoproteins provided a basic explanation of how high risk HPV types induce their oncogenic effects on cervical cells (Howley et al., 1991).

Although development of squamous cell carcinoma of cervix is strongly linked to infection by high risk HPV types but additional genetic or epigenetic changes also play an important role in carcinogenesis. Epigenetics is described as a heritable alteration in gene expression without a change in the DNA sequence (Egger et al., 2004). Epigenetics is a well-established phenomenon that plays a major role in various biological processes such as embryonic development, cancer biology, and immune system response, among many others (Gonzalez et al., 2005). In recent years, it has become apparent that epigenetic events are potentially equally
responsible for cancer initiation and progression as genetic abnormalities (Paluszczak and Wanda, 2006).

Epigenetic changes mainly include DNA methylation and histone modifications (Jablonka and Lamb, 2002; Egger et al., 2004), which are responsible for silencing important genes including those for tumor suppression, DNA repair and receptors. It has been proved that oncoproteins can possess the ability to modulate directly or indirectly the methylation machinery in order to silence cellular genes that could interfere with its tumor promoting actions. The Epstein-Barr virus oncogene product, latent membrane protein 1, induces down regulation of E-cadherin gene expression via activation of DNA methyltransferases (Tsai et al., 2002). In case of cervical cancer, the role of HPV genome DNA hypermethylation has only been the subject of study. One of the first indications of the importance of DNA methylation and viral gene expression came from studies of cell transfection with HPV-16-in-vitro methylated genomes, demonstrating that under these circumstances DNA is transcriptionally repressed (Rosl et al., 1993).

CpG islands are found in the promoter regions of many genes and their aberrant methylation in cancer cells leads to the functional silencing of those genes due to chromatin compaction. Abnormalities in DNA methylation have long been associated with cancer. Tumor suppressor genes like p53 can be inactivated not only through structural changes, like deletion, mutation etc. or by HPV- E6 but also by lack of expression through promoter hypermethylation (Gonzalez et al., 2005). Tumor suppressor gene epigenetic silencing is emerging as a well established oncogenic process.

Recent reports have uncovered the fact that some genes are found hypermethylated in preinvasive lesions, raising the possibility that testing for methylation of these genes may prove to be a useful screening tool (Narayan et al., 2003; Virmani et al., 2001; Zambrano et al., 2005), especially in cervical cancer as it evolves through a series of well-defined stages. A large percentage of cervical cancer cases are diagnosed at stage IV and their survival rate is less than 5%. Therefore, it is necessary to identify clinically useful early markers preferably using non-invasive
techniques. Methylated genes in serum of patients with cervical cancer can also be used as biomarkers (Anker and Stroun, 2002; Trejo-Becerril et al., 2003).

Cell cycle progression is triggered by the activation of cyclin-dependent kinase 4/6 (CDK4/6) upon binding of cyclin D, and further potentiated by subsequent activation of CDK2. On the other hand, the cell cycle is negatively regulated by the INK4 (p15, p16, p18 and p19) and the CIP/KIP (p21CIP1, p27KIP1 and p57KIP1) families of cyclin-dependent kinase inhibitors (CDKIs) (Malumbres and Barbacid, 2001). The INK4 family of CDKIs bind to and inhibit CDK4/6 (Lee and Yang, 2001). In contrast, the CDKIs of the CIP/KIP family may bind to both CDK2 and CDK4/6, and modulate their kinase activities.

The p14ARF gene on chromosome 9p is deleted and/or silenced by hypermethylation in a subset of human malignancies. There is evidence suggesting that p14 suppresses tumorigenicity by stabilizing the p53 protein (Shen et al., 2003). p14, a tumor suppressor gene, has been shown to be hypermethylated in various cancers like oral squamous cell carcinoma (OSCC) (Ishida et al., 2005). p16, located on chromosome 9p21, is the most commonly altered gene in human malignancies (Hirama and Koeffler, 1995). Aberrant methylation of p16 gene is observed in cervical cancer and it occurred very early within tumor cell populations in both in situ and invasive tumors at frequencies that varied from 10% to 100% (Virmani et al., 2001; Zambrano et al., 2005). Cyclin-dependent kinase inhibitor p15 gene is known to be inactivated by methylation in other tumors but no reports exist on cervical tumors (Gonzalez et al., 2005).

RARβ encodes retinoic acid receptor beta, a member of the thyroid-steroid hormone receptor superfamily of nuclear transcriptional regulators. The rate of RARβ2 methylation has been shown to progressively increase from 11% in low-grade to 29% in high grade lesions and 33-63% in invasive cancers (Narayan et al., 2003; Zambrano et al., 2005; Ivanova et al., 2002; Feng et al., 2005), suggesting that this abnormality is an early event in multistage cervical carcinogenesis. In cervical cancer, the RARβ2 gene is of particular interest because retinoic acid inhibits transformation of human
Introduction...

keratinocytes by HPV –16 (Khan et al., 1993). The human FHIT gene (fragile histidine triad), identified in 1996, is composed of 10 exons, of which five (exon5-9) encode the protein (Sozzi et al., 1996). The studies have confirmed that the FHIT gene is abnormally expressed in 30–78% of cervical dysplasia, carcinoma cell lines, and primary tumors (Ivanova et al., 2002; Narayan et al., 2003; Zambrano et al., 2005). In cervical cancer, DAP Kinase (DAPK) is methylated in up to 100% of cases (Narayan et al., 2003; Zambrano et al., 2005), which suggests that its loss of expression is needed for cervical cancer progression. p16, RARβ2 and DAPK have been shown to be hypermethylated in cervical cancer in different populations but no report exists on the hypermethylation of these genes in cervical cancer in north Indian population.

Signal Transducers and Activators of Transcription (STATs) are a family of transcription factors that play central roles in the responses of cells to cytokines, molecules that control every aspect of the immune system. STAT1 plays an important role in growth arrest, in promoting apoptosis and is implicated as a tumor suppressor. Silencing of the STAT1 gene via promoter methylation may contribute to squamous cell carcinoma of head and neck (SCCHN) tumor cell growth (Xi et al., 2006). Among the various tumor suppressor genes, p53 is more commonly undergoing changes in most human neoplasia than any other single gene reflecting its control of critical cellular activities (Greenblatt et al., 1994; Hussain et al., 2001). The hypermethylation status of the promoter region of p53 gene was detected in 3 of 26 cases in a study on breast carcinoma (Kang et al., 2001). Mutations in p53 gene are generally associated with majority of cancers and p53 promoter methylation has been studied in oral squamous cell carcinoma (OSCC) (Yeh et al., 2003). Since, cervical cancer is mainly squamous cell carcinoma, it is important to study the methylation status of these genes with respect to cervical cancer. The p73 gene is located on chromosome 1p36, a region frequently deleted in neuroblastoma, melanoma and breast cancer (Kaghad et al., 1997). A recent study found that epigenetic modification of p73 via CpG-island hypermethylation represents a critical alternative mechanism for inactivation of this gene in cervical cancer and high incidence of p73 hypermethylation (38.8%) in cervical cancer which was correlated with loss of its expression (Liu et al., 2004).
p21 gene is a downstream effector of p53 and belongs to the CIP/KIP family of cyclin dependent kinase inhibitors. According to several reports mutations or deletions of p21\textsuperscript{CIP1} has not been detected, but aberrant promoter hypermethylation might be considered as the most frequent way of transcriptional silencing of gene in most of cancers (Gartel and Tyner, 1999; Shiohara et al., 1994; Ying et al., 2004). p27 gene also belongs to this family and several reports have shown its inactivation or inhibition to be involved in tumor development (Ishida et al., 2005; Takashi et al., 2006). But, no report exists on methylation of these genes in cervical cancer.

Reactivation of tumor suppressor genes that have been silenced by promoter methylation is a very attractive molecular target for cancer therapy (Gonzalez et al., 2005). There are several demethylating agents currently being evaluated in preclinical and clinical studies. 5-aza cytidine and 5-aza-2-deoxycytidine are the most studied and were developed over 30 years ago as classical cytotoxic agents, but were subsequently discovered to be effective DNA methylation inhibitors. Two nucleoside analogs (azanucleosides) with hypomethylating activity, decitabine and 5-azacytidine, have recently been approved by the Food and Drug Administration (FDA) for the treatment of myelodysplastic syndrome (Silverman et al., 2002; Kantarjian et al., 2006). Clinical trials with these agents are ongoing in other type of cancers, for example, acute myeloid leukemia (AML) with encouraging results (Blum et al., 2005). However, toxicities (i.e., myelosuppression) inherent to the cell cycle phase specificity of nucleoside analogs pose significant limitations to the use of these drugs, especially in patients with solid tumors.

As most of these synthetic compounds may have cytotoxic effects, the focus is on natural products for the epigenetic reversal. Phytochemicals derived from fruits and vegetables, referred to as chemopreventive agents include genistein, resveratrol, diallyl sulfide, S-allyl cysteine, allicin, lycopene, capsaicin, curcumin, 6-gingerol, ellagic acid, ursolic acid, silymarin, anethol, catechins and engenol. These chemopreventive agents have potential to be used as adjuncts to current cancer therapies (Dorai and Aggarwal, 2004).
In the light of a few recent reports on the use of natural compounds for the reversal of epigenetic changes, various non-toxic dietary compounds and medicinal plant extracts can be screened for their ability to cause the reversal of the epigenetic silencing of tumor suppressor genes in the cancer cell lines.

The present study was undertaken with the following aims and objectives:

1.1 AIMS AND OBJECTIVES:

- To study promoter hypermethylation of tumor suppressor genes including p14, p15, p16, p21, p27, p53, and RARβ2 in cervical biopsies and serum samples of the cancer patients as compared to the age and sex matched controls.
- To study the correlation of the methylation status of the tumor suppressor genes with respect to stage of disease and HPV infection.
- Screening of dietary compounds and plant extracts for their potential to cause epigenetic reversal using different cervical cancer cell lines.