Cancer is responsible for one in eight deaths worldwide (Garcia M et al. 2007). It encompasses more than 100 distinct diseases with diverse risk factors and epidemiology which originate from most of the cell types and organs of the human body. Which are characterized by relatively unrestrained proliferation of cells that can invade beyond normal tissue boundaries and metastasize to distant organs. Much of our current understanding of cancer is based on the central tenet that it is a genetic disease, arising as a clone of cells that expands in an unregulated fashion because of somatically acquired mutations (Stratton et al. 2009). These somatic mutations include base substitutions, insertions and deletions (indels) of bases, rearrangements caused by breakage and abnormal rejoining of DNA, and changes in the copy number of DNA segments. They also often include epigenetic changes that are stably inherited over mitotic DNA replication, for example, alterations in methylation of cytosine residues (Laird 2005). Whether a mature cancer clone emerges in an individual person or not, is majorly influenced by environmental and life-style factors, as well as by the set of genomic sequence variants present in the fertilized egg from which the individual develops and that are therefore found in all somatic cells. These so-called constitutional or “germline” mutations can influence cancer susceptibility in a number of ways, including directly altering growth of the cancer clone, altering the mutation rate in somatic cells, or modulating the metabolism of carcinogens. Somatic mutations are thought to occur in the genomes of all normal cells as they proceed through the rounds of cell division that take place during development in-utero and during replenishment of body tissues in post-natal life. Additional somatic mutations continue to accumulate in cancer cells as they divide. The rate of acquisition and the types of somatic mutation that occur can be increased by exogenous and endogenous mutagenic exposures that cause DNA damage and are mitigated by DNA repair processes. Indeed, in the event that DNA repair fails, the somatic mutation rate may also increase (Stratton et al. 2009). The six hallmarks of cancer—distinctive and complementary capabilities that enable tumor growth and metastatic dissemination—continue to provide a solid foundation for understanding the biology of cancer (Fig. LF1).
All cancers are thought to share a common pathogenesis. Each is the outcome of a process of Darwinian evolution occurring among cell populations within the microenvironments provided by the tissues of a multi-cellular organism. Analogous to Darwinian evolution occurring in the origins of species, cancer development is based on two constituent processes, the continuous acquisition of heritable genetic variation in individual cells by more-or-less random mutation and natural selection acting on the resultant phenotypic diversity.

Nearly 12.7 million new cancer cases and 7.6 million cancer deaths occurred in 2008 worldwide; of these, 56% of the cases and 64% of the deaths occurred in the economically developing world. Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females, accounting for 23% of the total cancer cases and 14% of the cancer deaths. Lung cancer is the leading cancer site in males, comprising 17% of the total new cancer cases and 23% of the total cancer deaths. The ASR of men and women is shown in Fig. LF2.
Although overall cancer incidence rates in the developing world are half to those as seen in the developed world in both sexes, the overall cancer mortality rates are generally similar. Cancer survival tends to be poorer in developing countries, most likely because of a combination of a late stage at diagnosis and limited access to timely and standard treatment. The number of new cancer cases ranges from 3.7 million in Eastern Asia to about 1800 in Micronesia/Ponynesia (Wang, X. et al. 2008b). Cervical cancer is the third most common cancer in women, and the seventh overall, with an estimated 530 000 new cases in 2008. More than 85% of the global burden occurs in developing countries, where it accounts for 13% of all female cancers. India, the second most populous country in the world, accounts for 27% (72,825) of the total cervical cancer deaths.

Cervical cancer

Cervical cancer develops in the epithelial tissues of the cervix particularly at and around the transformation zone squamous cellular junction which refers to the place where the endocervix (the upper part close to the uterus which is covered by glandular cells), and the ectocervix (the lower part close to the vagina which is covered by squamous cells), meet (Fig.LF3).
It is well established that HPV infection is the main causal agent of cervical cancer (Walboomers et al. 1999; zur Hausen 2009). A key event in HPV infection that leads to development of cervical cancer is disruption of epithelial integrity at the site and binding of the virus to the cells of basement membrane (Doorbar 2006).

Cervical cancers are of several types, classified on the basis of where they develop in the cervix. Cancer that develops in the ectocervix is called squamous cell carcinoma, and around 80-90% of cervical cancer cases are of this type. Cancer that develops in the endocervix is called adenocarcinoma. In addition, a small percentage of cervical cancer cases are mixed versions of the above two, and are called adenosquamous carcinomas or mixed carcinomas. There are other types of cervical cancer, such as small cell carcinoma, neuroendocrine carcinoma etc. but these are rare. Cervical cancer begins with the development of pre-cancerous, benign lesions in the cervix area. The first stage in development is mild dysplasia, which can progress to moderate dysplasia, severe dysplasia, and then carcinoma in situ (CIS) or invasive cervical cancer. Mild dysplasia usually regresses on its own without treatment, and doesn’t progress to moderate or severe dysplasia. A small percentage of women with mild dysplasia, however, progress to more severe forms, although this can take as
long as 10 years. Women with moderate to severe dysplasia are at high risk of developing invasive cancer, although the progression from severe pre-cancerous lesions to cancer may take several years. Cervical cancer cells can spread by breaking away from the cervical tumor. They can travel through lymph vessels to nearby lymph nodes. Also, cancer cells can spread through the blood vessels to the lungs, liver, or bones. After spreading, cancer cells may attach to other healthy tissues and can grow to form new tumors that may damage those tissues.

**Epidemiology:** Cancer of the cervix uteri is the second most common cancer among women worldwide, with an estimated 529,409 new cases and 274,883 deaths in 2008. About 86% of the cases occur in developing countries, representing 13% of female cancers. Worldwide, mortality rates of cervical cancer are substantially lower than incidence with a ratio of mortality to incidence to 52% (Globocan 2008). This female malignancy alone accounted for 9% (529,800) of newly diagnosed cancer cases and 8% (275,100) of the total cancer deaths that occurred globally among females in 2008 (Globocan 2008). With an annual incidence of 132,000 and mortality rate of approximately 74,000, India disproportionately shares more than a quarter of the global cervical cancer burden. The major reason for high mortality from cervical cancer in India is late diagnosis owing to the lack of cervical screening and other preventive measures. Though cervical cancer has the highest prevalence among all cancers in India (the age-adjusted incidence 30.7 per 100,000 women), in past two decades all of the urban population-based cancer registries (PBCR) of ICMR have shown a significant decrease in the age-adjusted rates (AAR) of cervical cancer, whereas rural PBCR did not show such trend. The observed decline in the incidence of cervical cancer at some of the urban cities in the absence of any organized screening program has been ascribed to increasing awareness, late marriage, changing lifestyle and sexual behavior of the urban population.

**Types of cervical cancer**

There are several types of cervical cancer, classified on the basis of where they develop in the cervix (Lopez-Camarillo et al. 2012) (Lopez-Camarillo, Marchat et al. 2012) (Society 2009). There types are indicated below in the Table LT1.
Table LT1 - Types of cervical cancer

<table>
<thead>
<tr>
<th>Region of cervix</th>
<th>Type of cervical cancer</th>
<th>Occurrence of the type</th>
<th>Source of the cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectocervix</td>
<td>Squamous cell carcinoma</td>
<td>Most common (Around 80-90% of cervical cancer cases)</td>
<td>Squamous cells</td>
</tr>
<tr>
<td>Endocervix</td>
<td>Adenocarcinoma.</td>
<td>Less common than squamous cell cancer, but has become more common in recent years (about 10-15%).</td>
<td>Mucus-producing gland cells</td>
</tr>
<tr>
<td>Mixed versions of the above two</td>
<td>Adenosquamous carcinomas or mixed carcinomas</td>
<td>3-5%</td>
<td>Squamous and gland cells</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>Undifferentiated neoplasm</td>
<td>Rare (less than 3%)</td>
<td>The cells are smaller than normal cells, and barely have room for any cytoplasm</td>
</tr>
</tbody>
</table>

Classification of cervical cancer

The premalignant lesions or cervical dysplasia are well characterized and generally categorized as per disease severity using three system of classifications namely WHO, CIN and TBS (Table LT2).

Table LT2 - Different classification given for cervical lesions

<table>
<thead>
<tr>
<th>Classification systems</th>
<th>Proposed year</th>
<th>Diagnosis type</th>
<th>Transformation</th>
<th>Gradating of cervical lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO</td>
<td>1973</td>
<td>Cytological</td>
<td>Dysplasia</td>
<td>Carcinoma-in-situ</td>
</tr>
<tr>
<td></td>
<td>1975</td>
<td>Histological</td>
<td>Mild</td>
<td>Moderate</td>
</tr>
<tr>
<td>CIN</td>
<td>1969</td>
<td>Cytological</td>
<td>Premalignant</td>
<td>CIN-I CIN-II CIN-III</td>
</tr>
<tr>
<td>Bethesda system</td>
<td>1988</td>
<td>Cytological</td>
<td>LSIL</td>
<td>HSIL</td>
</tr>
</tbody>
</table>

The WHO classification was originally given by Riotton and Christoperson in 1973 for cytological diagnosis and by Poulsen and Taylor (in 1975) for histological diagnosis. According to WHO classification, cervical lesions can be classified into dysplasia, carcinoma-*in-situ* (CIS) and invasive cancer. Cervical dysplasia was further graded into mild, moderate and severe dysplasia. CIN classification was given by
Richart and Barron (1969) which suggested use of the term cervical intraepithelial neoplasia (CIN) to distinguish severe dysplasia from CIS. CIN is further classified into three groups namely CINI, CINII, CINIII which represents mild dysplasia, moderate dysplasia and severe dysplasia in combination with CIS, respectively of WHO classification. On the other hand, Bethesda classification was proposed by National Cancer Institute Workshop, to simplify the reporting of cervical and vaginal cytological diagnosis. In this system the precancerous lesions are termed as squamous intraepithelial lesions (SIL) and are further categorized in low grade SIL (LSIL) and high grade SIL (HSIL) (Solomon et al. 2002). LSIL encompasses cellular changes associated with HPVs and mild dysplasias or CINI whereas HSIL includes moderate dysplasia or CIN II, severe dysplasia and CIS as CINIII.

**Clinical staging of cervical cancer**

The process used to find out if cancer has spread within the cervix or to other parts of the body is called staging. The original staging for cervical carcinoma was introduced in 1928. Cervical cancer is staged by the International Federation of Gynecology and Obstetrics (FIGO) staging system, which is based on clinical examination, rather than surgical findings. It allows only the following diagnostic tests to be used in determining the stage: palpation, inspection, colposcopy, endocervical curettage, hysteroscopy, cystoscopy, proctoscopy, intravenous urography, and X-ray examination of the lungs and skeleton and cervical conization (Wikipedia). The American Joint Committee on Cancer (AJCC) TNM classification and the International Federation of Gynecology and Obstetrics (FIGO) staging system revised in 2009 for cervical cancer are provided below in Table LT3.
### Table LT3 - Cervical carcinoma clinical staging and their stage specific features.

<table>
<thead>
<tr>
<th>Clinical stages</th>
<th>Specific features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>The carcinoma is confined to the surface layer (cells lining) of the cervix. Also called carcinoma in situ (CIS).</td>
</tr>
<tr>
<td>Stage I</td>
<td>Carcinoma strictly confined to the cervix; extension to the uterine corpus should be disregarded. The diagnosis of both Stages IA1 and IA2 is based on microscopic examination of removed tissue, preferably a cone, which includes the entire lesion.</td>
</tr>
<tr>
<td>Stage IA</td>
<td>Invasive cancer identified only microscopically. Invasion is limited to measured stromal invasion with a maximum depth of 5 mm and no wider than 7 mm.</td>
</tr>
<tr>
<td>Stage IA1</td>
<td>Confined to the cervix, diagnosed only by microscopy with invasion of &lt; 3 mm in depth and lateral spread &lt; 7 mm</td>
</tr>
<tr>
<td>Stage IA2</td>
<td>Confined to the cervix, diagnosed with microscopy with invasion of &gt; 3 mm and &lt; 5 mm with lateral spread &lt; 7 mm</td>
</tr>
<tr>
<td>Stage IB</td>
<td>Clinical lesions confined to the cervix or preclinical lesions greater than Stage IA. All gross lesions even with superficial invasion are Stage IB cancers.</td>
</tr>
<tr>
<td>Stage IB1</td>
<td>Clinically visible lesion or greater than A2, &lt; 4 cm in greatest dimension</td>
</tr>
<tr>
<td>Stage IB2</td>
<td>Clinically visible lesion, &gt; 4 cm in greatest dimension</td>
</tr>
<tr>
<td>Stage II</td>
<td>Carcinoma that extends beyond the cervix, but does not extend into the pelvic wall. The carcinoma involves the vagina, but not as far as the lower third.</td>
</tr>
<tr>
<td>Stage II A1</td>
<td>Involvement of the upper two-thirds of the vagina, without parametral invasion, &lt; 4 cm in greatest dimension</td>
</tr>
<tr>
<td>Stage II A2</td>
<td>&gt; 4 cm in greatest dimension</td>
</tr>
<tr>
<td>Stage II B</td>
<td>With parametral involvement</td>
</tr>
<tr>
<td>Stage III</td>
<td>Carcinoma that has extended into the pelvic sidewall. On rectal examination, there is no cancer-free space between the tumour and the pelvic sidewall. The tumour involves the lower third of the vagina. All cases with hydronephrosis or a non-functioning kidney are Stage III cancers.</td>
</tr>
<tr>
<td>Stage III A1</td>
<td>No extension into the pelvic sidewall but involvement of the lower third of the vagina.</td>
</tr>
<tr>
<td>Stage III A2</td>
<td>Extension into the pelvic sidewall or hydronephrosis or non-functioning kidney.</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Carcinoma that has extended beyond the true pelvis or has clinically involved the mucosa of the bladder and/or rectum.</td>
</tr>
<tr>
<td>Stage IV A1</td>
<td>Spread of the tumour into bladder or rectal wall and adjacent pelvic organs.</td>
</tr>
<tr>
<td>Stage IV A2</td>
<td>Spread to distant organs such as abdomen, liver, intestinal tract or lungs.</td>
</tr>
</tbody>
</table>

### Grading of cervical cancer:

Grading is a way of classifying cancer cells based on their appearance and behavior when viewed under a microscope. To find out the grade of a tumor, the biopsy sample is examined under a microscope. A grade is given based on how the cancer cells look and behave compared with normal cells (differentiation). This can give the healthcare team an idea of how likely the cancer is to spread. The grade of
cervical cancer is based on the degree of differentiation of cells and their rate of growth. Grading plays an important part in planning cervical cancer treatment and can also be used to help estimate the prognosis (future outcome). Different types of grades of cervical cancer are defined below in Table LT4.

**Cervical cancer symptoms**

The most common symptom of cervical cancer is bleeding from the vagina at other times than when a woman is having a period. The woman may have bleeding:

- Between periods
- After or during sex
- At any time if a woman is past her menopause

Some women also have:

- A vaginal discharge that smells unpleasant
- Discomfort or pain during sex (Cancer Research UK 2011)

**Risk factors and co-factors of cervical cancer**

A number of risk factors/cofactors are likely to be involved in the development of cervical cancer. The major category of risk factors consists of mainly infection with high risk HPV types and viral factors that influence magnitude and duration of HPV infection such as, persistence of infection, co-infection of multiple HR-HPV types, HR-HPV variants, viral load and physical state of viral genome into host genome. Binding of HR-HPV E7 to pRB activates the E2F transcription factor, which triggers the expression of proteins necessary for DNA replication (Munger et al. 2004). Unscheduled S-phase would normally lead to apoptosis by the action of p53; however, in HPV-infected cells, this process is counteracted by the
viral E6 protein, which targets p53 for proteolytic degradation (Thomas et al. 1999). As a consequence, the dependence on cell cycle control is abolished and normal keratinocyte differentiation is retarded (Doorbar 2005). As an aberration of virus infection, constant activity of the viral proteins E6 and E7 leads to increasing genomic instability, accumulation of oncogene mutations, further loss of cell-growth control, and ultimately cancer (Duensing and Munger 2004).

HUMAN PAPILLOMA VIRUS (HPV)

HPVs belong to a family of small, closed, circular, double stranded DNA viruses consisting of more than 200 related genetic types, of which more than 85 are fully sequenced (Burd 2003). The papillomaviridae family represents a heterogeneous group of viruses with a remarkable diversity of tissue tropism, prevalence and pathological outcome. These viruses are epitheliotropic in nature, infect mucosal and cutaneous epithelial tissues and preferentially localize in the anogenital tract. HPV E6 and E7 are the principal transforming genes (Yutsudo et al. 1988). Of the approximately 30 HPVs that infect the anogenital tract, 15 HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82) associate with high grade lesions and invasive cancer and hence are designated as “high-risk” types (Shukla et al. 2009b). Among these, HPV types 16 and 18 are the two common highly oncogenic types. Prevalence of other high-risk types is very low and rarely high in specific endemic populations or they appear as co-infections to HPV 16 and 18. Other 11 different HPV types (HPV 6,11,40,42,43,44,54,61,70,81,CP6108) are termed as “low-risk” types and are associated with genital warts and benign cervical lesions. HPV6, 11 cause approximately 90% of genital warts. Majority of HPV infections are transient and subclinical and undergo subsequent clearance by the immune system. Persistence of HPV infection results in development of genital warts as well as precancerous lesions which progress to cancers of uterine cervix. Molecular studies have revealed the presence of cervical HPV infection, which ranges up to 10-12% in normal population, upto 65% in low-grade squamous intraepithelial lesions (SILs), 86% in high-grade SILs, 83% in adenocarcinomas and 98% in squamous cell carcinoma (SCC) of the cervix. In India, approximately 90% of invasive cervical cancer cases are SCC, while 8-10% are adenocarcinomas. While global reports indicate preferential occurrence of HPV16 in SCC and HPV18 in adenocarcinoma, In
India, HPV16 is the most prevalent type both cervical cancer types. In a similar contrast HPV16 and HPV18 collectively represent approximately 70% of total HPV infection in majority of the countries, the prevalence of HPV16 in India is found to be exclusively high and ranges upto 90%, while prevalence of HPV18 varies from 3-20% followed by other high risk types such as HPV45, 33, 35, 58, 59, 31, 56, 51, 52, 73, 62, 64, 39, 61, 68, and 82 in decreasing order of prevalence. In a national HPV mapping study in India, prevalence of HPV16 was found to be highest in Chennai (88%), and lowest in Jammu and Kashmir (14.2%).

**GENOME ORGANIZATION OF HPV**

Genomic organization is a well conserved among papillomaviruses (Fig. LF4). All HPV type contains a double stranded closed-circular DNA genome of 7200-8000 base pairs which is organized into three main regions: two protein coding regions (early and late) and a non-coding region LCR. They are non-enveloped virions with icosahedral capsid composed of 72 capsomeres with a star shaped morphology and displaying a cylindrical channel along their axis (Pfister and Fuchs 1994). HPV particles are about 55nm in diameter (Klug and Finch 1965). This non-enveloped structure renders them relatively resistant to heat and organic solvents (Bonnez 1993). A peculiar characteristic of all known HPV types is that all putative protein coding sequences are restricted to one strand of the genome (Pfister and Fuchs 1994).

![Figure LF4- HPV16 genomic organization and functions of viral gene products.](image)

E1, E2, E3, E4, E5, E6 and E7 are early genes. E6 and E7 are required for establishment, viral replication and maintenance of viral oncogenic activity. L1 and L2 are late capsid proteins.
FUNCTIONS OF HPV GENES

URR REGION: The URR possesses numerous binding sites for many repressors and activators of transcription, suggesting that it may play a part in determining the range of hosts for specific HPV types (Turek 1994).

E1 AND E2: Encode proteins that are vital for extra-chromosomal DNA replication and the completion of the viral life cycle. The E2 also encodes two proteins: one, which inhibits transcription of the early region; and the other, which increases the transcription of the early region (Ward et al. 1989). A hallmark of HPV-associated cervical carcinoma is loss of the expression of the viral E2 protein (Thierry et al. 2004).

E4: The E4 protein is expressed in the later stages of infection when complete virions are being assembled, and is not known to have transforming properties, however it is considered to play an important role for the maturation and replication of the virus (Brown et al. 1994).

E5: The E5 in open reading frame (ORF), meanwhile, is often deleted in cervical carcinoma cells, indicating that it might not be essential in maintaining the malignant transformation of the host cell. When present, E5 interacts with various trans-membrane proteins like REGF, PDGF β and colony stimulating factor (Hwang et al. 1995).

E6 AND E7: In the protein-encoding regions, the E6 and E7 ORF are considered to play the most major roles. These two units encode for oncoproteins that allow replication of the virus and the immortalization and transformation of the cell that hosts the HPV DNA (Bedell et al. 1987; Phelps et al. 1988) (Spitkovsky et al. 1996). The HPV E6 gene product binds to p53 and targets it for rapid degradation via a cellular ubiquitin ligase. The HPV E7 gene product binds to the hypo-phosphorylated form of the RB family of proteins.

L1 AND L2: The late region units, L1 and L2 encode for viral capsid proteins during the late stages of virion assembly (Park et al. 1995). The minor capsid protein encoded by L2 has more sequence variations than that of the L1 protein (Motoyama et al. 2004).

Upstream Regulatory Region (URR)/ Long Control Region (LCR): Viral late and early genes are separated by a transcriptional control region designated URR or LCR.
or the non-coding region. This region is approximately 800-1000bp (covering about 10% viral genome). It does not encode any protein, but contains the origin of replication, viral promoter and enhancer sequences and plays a central regulatory role in HPV-associated cell transformation and viral life cycle.

The promoter of E6/E7 transcription is located at the 3’ end of the URR. The URR region of the papillomavirus contains enhancer elements that are responsive to the cellular factors as well as to virally encoded transcription regulatory factors. All papillomavirus URRs studied so far contain epithelial specific constitutive enhancers (Sailaja et al. 1999) that contribute to the epithelial tissue tropism of HPVs. Most HPV-types have a promoter in front of the E6 gene in common (Thierry et al. 1987; Stubenrauch et al. 1992). For HPV-16, P97 is the major promoter which directs the expression of E6 and E7 as well as several other early gene products. Functionally, the 850 bp HPV 16 URR can be divided into three parts:

1). A 5’-terminal portion of unknown function, which only marginally contributes to the activity of E6/E7 promoter.

2). A central 400bp constitutive enhancer essential for E6/E7 promoter activity,

3). A promoter proximal region containing E6/E7 promoter p97 at its 3’ end.

Several factors controls the HPV transcription such as multiple promoters, multiple splice pattern, different polyadenylation signals and several proteins and factors produced by the virus as well as the host. Because of its potential for binding with a wide array of specific transcription factors, URR plays a critical role in determining the host range of specific types of HPVs (Cripe et al. 1987). Within LCR cis-acting elements regulate transcription of E6/E7 genes

**Life Cycle of HPV**

The HPV life cycle consists of initial infection, uncoating, genome maintenance, genome amplification and packaging to form new viral particles. Most of the work in this area and its associated genetic events has focused on HPV type 16 (HPV16) which is a major cause of cervical cancer (Malik 2005). The host tissue of human papillomaviruses is the stratified epithelium. This tissue is complex in that it is composed of layered sheets of non-dividing cells in various stages of terminal
differentiation, with the uppermost layer being the most differentiated. Only cells of
the bottom-most layer of this tissue, the basal cells, proliferate. Although the HPV life
cycle begins with the infection of a basal cell, it only comes to completion when the
infected cell reaches the upper layers of the epithelium. Thus life cycle of HPV can be
divided into four phases.

**Infection and un-coating (viral entry into host cells):** Infection by papillomaviruses
requires that virus particles gain access to the epithelial basal layer and enter the
dividing basal cells which may be facilitated by micro traumas and micro-abrasions. It
has also been suggested that for a lesion to be maintained, the virus must infect an
epithelial stem cell (Schmitt et al. 1996). At present there is some controversy as to
the precise nature of the receptor for virus entry (Patterson et al. 2005) but it is
thought that heparin sulphate proteoglycans may play a role in initial binding and/or
virus uptake (Joyce et al. 1999; Shafti-Keramat, 2003 #24; Shafti-Keramat et al.
2003; Patterson et al. 2005). As with other viruses (Chung, C. S. et al. 1998;
Summerford et al. 1999), it seems that HPV infections requires the presence of
secondary receptors for efficient infection, and it has been suggested that this role
may be played by the α6 integrin (Evander et al. 1997; McMillan et al. 1999; Bossis
et al. 2005). Papillomavirus particles are taken into the cell relatively slowly
following binding (Culp and Christensen 2004) and for HPV16, this occurs by
clathrin-coated endocytosis (Day et al. 2003), while HPV31 gain entry via caveolae
(Bousarghin et al. 2003).

**Genome maintenance (viral genome status):** Infection leads to the establishment of
the viral genome as a stable episome (without integration into the host cell genome) in
cells of the basal layer, and this is thought to require expression of the viral replication
proteins, E1 and E2. E2 binding to the viral LCR is necessary for the recruitment of
the E1 helicase to the viral origin, which binds in turn to cellular proteins necessary
for DNA replication, including RPA (replication protein A) and DNA polymerase α
primase (Masterson et al. 1998; Conger et al. 1999; Loo and Melendy 2004). In the
basal cells, it appears that the viral genome replicates with the cellular DNA during S-
phase, with the replicated genomes being partitioned equally during cell division. The
role of E2 in anchoring viral episomes to mitotic chromosomes is critical for correct
segregation (You et al. 2004) and, in some papillomavirus types, involves the cellular
Brd4 protein, which directly associates through its C-terminus with the viral E2 protein (You et al. 2004; McPhillips et al. 2005).

**Transcriptional Control: Regulation of Viral Oncogene Expression**

All papillomaviruses have in common that transcription of early and late genes occurs uni-directionally from one or several promoters located in the LCR or in the E6 and E7 genes. All transcripts undergo differential splicing which processes seven to nine genes of papillomaviruses to numerous different polycistronic mRNAs. In general, all papillomaviruses contain an E6 promoter that is activated by a TATA box and a Sp1 binding site. An enhancer, which activates transcription especially in epithelial cells, is centered about 400 bp upstream of E6 promoter. A silencer modulated by host cell factors like YY1 and CDP, is positioned between the enhancer and promoter and is important for HPV transcription in differentiating epithelium. The HPV DNA exists in the nucleus of infected cells in the form of chromatin, i.e. wrapped around nucleosomes. This nucleosomal structure determines the accessibility of cis-responsive elements and is modulated by histone acetylation and deacetylation which is mediated by some of the host cell factors binds to the enhancer and silencers sequences within viral LCR. The interaction between the different host cell regulatory factors at different regulatory levels of epithelial differentiation during course of HPV infection is likely to be a major source of alternative outcomes of HPV infections. The central point of attention in all these regulatory processes of viral life cycle is presence of HPV LCR which provides docking sites for all type of regulatory elements. The detailed description of characterized viral regulatory sequences, promoter structure, and viral transcription regulation is given below:

*Structure and function of the HPV early promoter:* The early promoter was named after the genomic sequence with numbering corresponding to the 5′ end of the E6 and E7 messenger RNA which are nucleotide 105 for HPV18 and 97 for HPV16 and HPV31 for instance. As expected for RNA Polymerase II promoters, a consensus TATA box is present in the viral promoter, upstream of the transcription initiation site that recruits the TFIID general transcription factor. Upstream sequences of this TATA box contain a transcriptional enhancer to which cellular transcription factors bind (Gloss et al. 1987; Swift et al. 1987; Garcia-Carranca et al. 1988). Sequences of these enhancers and the transcription factors involved are only partly conserved.
among mucosal HPVs, although these viruses exhibit the same host restriction. Among elements distinctively conserved in the mucosal HPV genomes are the sequences and locations of the binding sites for the viral E2 proteins. There are four of them distributed in the regulatory regions at comparable locations upstream of the initiation site, with two tandem repeated sites located 3 or 4 nucleotides upstream of the TATA box. This unique distribution of the E2 binding sites in the viral regulatory regions is a landmark of mucosal HPV whereas in other papillomaviruses their numbers and locations vary (Sanchez et al. 2008)

Host Cell Transcription Factors Relevant for Control of LCR Activity

Transcription factors are proteins involved in the regulation of gene expression that bind to the promoter elements upstream of genes and either facilitate or inhibit transcription. Through this process they control and regulate gene expression. Transcription of E6 and E7 is regulated by the LCR which is preserved in almost all cervical carcinoma cells. The URR regions of different HPV types has a number of binding sites that are shared among all types as well as some that are unique (Fig. LF5).

A large number of cellular transcription factors have been identified which binds to the frequently studied high-risk HPVs LCR: among them NF-1, AP1, KRF-1, Oct-1, SP-1, YY-1, STAT3 and the glucocorticoid receptor (Mack and Laimins 1991; Bauknecht et al. 1992; Hoppe-Seyler and Butz 1992; Thierry et al. 1992; Hoppe-Seyler and Butz 1994) appears to play a significant role in papillomavirus-linked carcinogenesis by regulating HPV transcription. Most of these factors bind to
the central region of the LCR, the *enhancer-region*. They regulate the transcription of the E6/E7 promoter located at the 3'-terminus of the LCR. Although many of these factors stimulate the promoter, some of them (specifically YY-1) have a dual function and repress and stimulate the viral promoter (*Bauknecht et al. 1992; Bauknecht et al. 1995*).

**Activator Protein 1 (AP-1) and regulation of HPV transcription**

Although the AP-1 (activating protein-1) transcription factor was identified almost 2 decades ago (*Angel et al. 1987; Lee, W. et al. 1987*) and retroviral homologs of some of its components were found even earlier, the biological relevance and physiological functions of AP-1 and its components are still being elucidated.

Tumorigenesis is multistep process, which involves cell transformation, invasive growth, angiogenesis and tumor spread to distant sites. Several of the AP-1 proteins such as c-Fos, FosB and c-Jun can transform cells efficiently in culture. When widely over expressed in mice, c-Fos causes osteosarcoma formation by the transformation of chondroblasts and osteoblasts (*Grigoriadis et al. 1993*).c-Jun is more important in the development of skin and liver tumors as conditional inactivation of c-jun in the liver interferes with the development of chemically induced papillomas and liver tumors (*Young et al. 1999; Eferl et al. 2003*). Over expression of Fra-1 and Fra-2 in transgenic mice leads to the development of lung tumors and epithelial tumors respectively (*Eferl and Wagner 2003*).

Both the HPV16 URR and the HPV18 URR are bound by AP-1 transcription factors, which specifically bound to its *cis* - regulatory DNA elements consisting of consensus sequence TGAGTCA. The transcription factor AP-1 is constitutively activated during human cervical cancer progression. c-Fos overexpression is associated with the pathoneogenesis of invasive cervical cancer (*Cheung et al. 1997*). The AP-1 binding activity is very high in cervical tumor tissues, whereas it is nil or, negligible in normal cervical cells. This strengthens the previous hypothesis that the activator protein, AP-1 is not only the major determinant for tissue specificity (*Offord and Beard 1990*) but also absolutely indispensable for efficient HPV oncogene expression (*Rosl et al. 1997*); (*Prusty et al. 2005*).
Signal Transducer and activator of Transcription (STAT3)

Signal transducer and activator of transcription (STAT3) is one of the members of a family of transcription factors. It was first identified in 1994 as a DNA-binding factor that selectively binds to the IL-6-responsive element (Akira et al. 1994). STAT-3 was also independently identified as a DNA-binding protein in response to epidermal growth factor (Zhong et al. 1994). The gene that encodes STAT-3 is located on chromosome 17q21. The 92-kDa protein is 770 amino acids long with sequential N-terminal coiled-coil domain, DNA-binding domain, a linker, SH2 domain, and C-terminal transactivation domain. The latter contains a tyrosine residue at position 705 and a serine residue at position 727, which undergoes phosphorylation when activated (Aggarwal et al. 2009). A growing number of human malignancies and tumour formation are associated with high levels of activation of STATs, very frequently STAT3 and Stat5 (Garcia et al. 1997; Garcia and Jove 1998; Bowman et al. 2000). STAT3, as a major member of the STAT family consisting of Stat1, Stat2, STAT3, Stat4, Stat5a, Stat5b, and Stat6, plays important roles in cell differentiation and proliferation (Darnell et al. 1994; Schaefer et al. 1995; Buettner et al. 2002).

In a variety of human cancers, constitutive activation of STAT3 is sufficient to induce tumour formation. The constitutive activation of STAT3 is frequently detected in a variety of human cancers. STAT3 has been classified as a proto-oncogene because an activated form of STAT3 can mediate oncogenic transformation (Bromberg and Darnell 1999; Buettner et al. 2002).

Activation of STAT3

STAT-3 is activated by many cytokines and growth factors, including epidermal growth factor (Cao et al. 1996), platelet-derived growth factor4 and IL-61 as well as by oncogenic proteins, such as Src(Yu et al. 1995) and Ras6 (Giordano et al. 1997). In addition numerous carcinogens, such as cigarette smoke (Arredondo et al. 2006) and tumor promoters, have been identified that can activate STAT-3 (Tharappel et al. 2002; Chan, K. S. et al. 2004). The activation of STAT-3 is regulated by phosphorylation of tyrosine 705 by receptor and non-receptor protein tyrosine kinases. These include epidermal growth factor receptor (EGFR) kinase (Garcia et al. 1997), Src (Yu et al. 1995), JAK (Lutticken et al. 1994; Migone et al.
The phosphorylation of STAT-3 in the cytoplasm leads to its dimerization, with subsequent translocation into the nucleus, and DNA binding, as a result genes that regulate cell proliferation, differentiation, and apoptosis are expressed. In addition, numerous serine Kinases have been implicated in the phosphorylation of STAT-3 at serine 727. These include protein kinase C (PKC) (Jain et al. 1999), mitogen-activated protein Kinases (MAPK), and CDK5 (Fu et al. 2004). PKC-ε has been shown to interact with STAT-3 directly and phosphorylated serine 727 (Aziz et al. 2007), which maximizes its transcriptional activity (Wen and Darnell 1997; Yokogami et al. 2000).

**Downstream targets and negative regulators of STAT3**

The recognition of STAT proteins as being potential players in oncogenic cell transformation was paralleled by their involvement in the modulation of activity for both promoters of MMPs and tissue inhibitors of MMP (TIMPs) (Gatsios et al. 1996; Korzus et al. 1997; Vincenti et al. 1998; Catterall et al. 2001). The STAT3 signaling pathway regulates cancer metastasis by regulating the expression of MMP-2 and MMP-9 genes that are critical to invasion and angiogenesis (Huang 2007). STAT3 signaling directly regulates MMP-2 expression, tumor invasion and metastasis, and STAT3 activation might be a crucial event in the development of metastasis (Xie et al. 2004). Direct interaction of STAT3 has been reported with the MMP-2 promoter since 7 putative Stat-binding elements have been identified in the MMP-2 promoter region (Xie et al. 2004). STAT3 has also been required for the induction of MMP-9 in transformed human mammary epithelial cells (Dechow TN, 2004). STAT3 can activate the MMP-9 promoter in breast cancer cell lines (Song, Y. et al. 2008).

STAT-3 activation is negatively regulated through numerous mechanisms. These involve the SOCS (Starr et al. 1997), PIAS (Chung, C. D. et al. 1997), protein phosphatases (Stahl et al. 1995), and ubiquitination-dependent proteosomal degradation (Daino et al. 2000). The SOCS proteins were shown to bind to the JAK activation loop as pseudo-substrate inhibitors through their SH2 domain, thereby blocking subsequent signaling that requires phosphorylation and activation of STAT-3 (Zhang et al. 1999). In contrast to SOCS, the PIAS-3 are nuclear factors that are
able to interact with phosphorylated STAT-3 and block transcription (Chung, C. D. et al. 1997). Smad4 has been shown to suppress the tyrosine phosphorylation of STAT-3 in pancreatic cancer cells (Zhao, S. et al. 2008). STAT-3 activation is also negatively regulated by various protein tyrosine phosphatases, including CD45 (Irie-Sasaki et al. 2001), PTEN (Sun and Steinberg 2002), SHP-1 (Migone et al. 1998), SHP-2 (Schaper et al. 1998).

STAT3 in cervical cancer

Although HPV infection is essential, it is not sufficient for ultimate tumorigenic transformation and requires certain crucial host cell factors to regulate its viral gene transcription. Expression of viral transforming genes, E6 and E7, of HR-HPVs is primarily regulated by cis-element rich enhancer region termed as LCR(8), Apart from viral transcriptional regulator, E2, the expression of viral genes/oncogenes is controlled by host’s sequence-specific transcription factors such as SP-1, AP-1 and NF-kB, that specifically bind to the LCR (Thierry 2009). These transcription factors are normally modulated at the level of expression and/or their activation. Host transcription factors in association with viral factors are likely to dictate viral latency, vegetative replication or oncogenic transcription during HPV infection (Prusty and Das 2005). Aberrant activation of STAT3, a member of STAT family, has been strongly associated with carcinogenesis and shown to promote cell cycle progression, cell proliferation and oncogenic transformation (Kim, D. J. et al. 2007). Though some studies demonstrate presence of STAT3 in a subset of cervical lesions (Yang, S. F. et al. 2005; Yang, S. F. et al. 2006; Chen, C. L. et al. 2007; Takemoto et al. 2009), not much is known about the expression and activation of STAT3 during cervical carcinogenesis in general, and its relation to HPV infection, in particular. Recently, a potential STAT3 binding site has been mapped on to 5’ region of HPV16 LCR that controls expression of viral oncogenes (Arany et al. 2002), thus suggesting a plausible productive interaction between HPV infection and STAT3 signaling (Shukla et al. 2010).

**STAT3 as an oncogene:** STAT3 in particular can be considered an oncogene, since the constitutively activated mutant, STAT3C, is capable of inducing tumors in nude mice (Bromberg and Darnell 1999). However, since no STAT3 mutation has been detected in human tumors so far, this statement could be challenged. The mutations
can affect the genes promoter and cause protein over-expression, but they can also affect the coding region and generate a hyper-activated mutant. As yet, none of these have been reported for STAT3. Rather, its activation in malignancies seems to occur downstream of hyper-activated tyrosine kinases and other types of oncogenes. However, STAT3 and STAT5 activate cyclinD1, c-Myc, and Bcl-xL, and it is generally accepted in the field that STAT3 promotes cell growth and therefore oncogenic processes. Hence, even -though not being a bona fide oncogene, but because of its direct activation by other oncogenic factors and its role in growth stimulation, STAT3 can still be considered an appropriate target for cancer treatment.

**STAT3 as a Target for Cancer Treatment:** The transcription factors are the final switches that activate gene expression patterns leading to malignancy. It is therefore logical to choose these proteins as anticancer targets, since targeting a single transcription factor can block the effects of a multitude of upstream genetic aberrations leading to cancer. A gene-therapy vector that was designed to inhibit STAT3 signaling in a mouse model of murine melanoma has provided proof of concept that human STAT3 is a valid target for cancer therapy (Niu et al. 1999). In this study, the expression of a dominant-negative form of STAT3 induced massive apoptosis in mouse melanomas. An unexpected side effect of targeting STAT3 was the accompanying bystander effect: adjacent tumor cells also underwent apoptosis without having undergone gene therapy. These studies were performed in immune-competent mice using a syngeneic mouse-melanoma cell line. Therefore, this potent bystander effect might involve the immunosuppressive activity of STAT3 (Wang, T. et al. 2004). However, it might also reflect the importance of STAT3 signaling for angiogenesis, or for the release of soluble factors capable of inducing apoptosis. Given the similarities between STAT3 and STAT5, it is worth investigating whether gene therapy of STAT5 dependent tumors triggers a similar bystander effect.

Finally, tissue-specific ablation of STAT3 has shown that non-oncogenic cells lacking STAT3 proliferate and survive well in vitro and in vivo (Akira 2000). Furthermore, inhibition of STAT3 in mouse fibroblasts does not have deleterious effects in normal cell growth (Turkson et al. 1998; Niu et al. 1999). The selective response to STAT3 inhibitors in tumor versus normal cells might reflect that tumor cell growth and survival irreversibly depend on high levels of activated STAT3, whereas normal cells might be able to withstand lower levels or use alternative
pathways. This is an ideal property of a therapeutic target, since it could imply that STAT3 inhibitors should not affect normal cells. A specific inhibitor for STAT3 could therefore be very useful for the treatment of cancer. Overall, STAT3 seems to be the most promising candidate of the family, not only due to its activation in many tumors, but also because of its immunosuppressive activity (Wang, T. et al. 2004). Its inhibition could slow down the proliferation of oncogenic cells, as well as stimulate antitumor immunity, which should therefore result in a potent reduction of tumor growth.

**microRNAs biogenesis and functions**

MicroRNAs are short RNA molecules of 19–25 nucleotides in length which were recently identified to play a key role in regulating gene expression by inhibiting translation and/or triggering degradation of their target mRNAs (Chen, C. Z. et al. 2004). MicroRNA genes are generally transcribed by RNA Polymerase II (Pol II) in the nucleus and are exported to the cytoplasm as mature miRs. A schematic presentation of miRs biogenesis is given in Figure LF6. The mature miRNA binds to complementary sites in the mRNA target to negatively regulate corresponding gene expression. The miR negative regulatory action is executed in one of the two ways that primarily depends on the degree of complementarity between the miRNA and its target mRNA. miRNAs that bind to the 3’ UTR of their target mRNA genes with imperfect complementarity block target gene expression at the level of protein translation. miRNAs that bind in the coding sequence or open reading frame (ORF) of their mRNA target with perfect (or nearly perfect) complementarity induce target-mRNA cleavage.

miRs are important players in regulation of various biological processes including cell differentiation, proliferation and apoptosis (Arora et al.)(miRBase release 18 (published in November, 2011) contains 18226 entries representing hairpin precursor miRNAs, expressing 21643 mature miRNA products, in 168 species (Arora et al.)(http://www.mirbase.org/). There are 1527 precursors and 2154 mature unique human miRNAs have been discovered till now. Over 50% of mammalian miRNAs are located within the intronic regions of annotated genes (Rodriguez et al. 2004), suggesting that the expression of these miRNAs might be controlled by the promoters of their host genes.
Figure LF6: Brief outline of biogenesis of functional miRs. MicroRNA genes transcribed by RNA Polymerase II (Pol II) are large pri-miRNA transcripts of several kilobases in size, capped and polyadenylated. pri-miRNAs are processed by the RNase III enzyme Drosha and its co-factor, Pasha, to release ~70-nucleotide pre-miRNA precursor. RAN–GTP and exportin-5 transport the pre-miRNA from nucleus into the cytoplasm. RNase III enzyme, Dicer, processes the pre-miRNA to generate a transient ~22-nucleotide miRNA:miRNA* duplex and makes it ready for loading into the miRNA-associated Argonaute containing multiprotein RNA-induced silencing complex (RISC). Mature single-stranded miRNA is formed following cleavage of complementary strand which subsequently binds to complementary sites in the mRNA target and negatively regulates gene expression. The miR negative action mechanism is one of two ways that depend on the degree of complementarity between the miRNA and its target mRNA. miRNAs that bind to the 3’ untranslated regions (3’ UTRs) of their target mRNA genes with imperfect complementarity block target gene expression at the level of protein translation. miRNAs that bind with perfect (or nearly perfect) complementarity to the coding sequence of their mRNA target induced cleavage of the target-mRNA.

MicroRNAs and cancer

Expression of miRNAs is altered in a number of human diseases from psychiatric disorders (Maes et al. 2009) to cancer (He et al. 2005). Because of being an upstream regulatory molecule with multiple targets, the changes in levels of miR expression are anticipated to be the cause of multiple alterations seen in mRNA and protein profiles during carcinogenesis. Studies have revealed that miRNAs frequently reside within fragile sites, are often involved in cancer development (Calin et al. 2004). However, it has remained a puzzle whether altered miRNA expression is a cause or consequence of carcinogenic processes. The molecular basis of miRNA-
mediated gene regulation is not fully understood and their role in tumorigenesis remains largely unknown. Emerging data shows appearance of alterations in a limited set of specific miRNAs in many cancer types indicates to a potential regulatory role of these miRNAs in carcinogenic process and could be a reflection of dynamic state between tumor suppressors and oncogenic oncomiRs.

A. miRNAs as tumor suppressors

The first link between miRNAs and cancer came from the study that found that 13q14 locus that contain miR-15 and miR-16, is frequently deleted in B-cell chronic lymphocytic leukemias (Calin et al. 2002). Thereafter, miR-125b-1 which is located in a fragile site on chromosome 11q24, was shown to be deleted in a subset of patients with breast, lung, ovarian and cervical cancers (Calin et al. 2004). Subsequent studies repeatedly showed a strong correlation between abrogated expression of certain specific miRNAs and oncogenesis. Among these, miR-143 and miR-145 were found significantly reduced in colorectal tumours, breast, cervical and prostate cancer (Michael et al. 2003; Iorio et al. 2005; Wang, X. et al. 2008b; Peng et al. 2011). The absence of miRLet-7 results in failure in induction of cell cycle exit and terminal differentiation in adult stem cells (Reinhart et al. 2000), whereas overexpression of Let-7 in lung adenocarcinoma cell line inhibited lung cancer cell growth in vitro (Takamizawa et al. 2004). These studies indicate that miRNAs like miR-15, miR16, miR-125-b-1 and Let-7 could have growth suppressing properties and targeted expression of these miRs in cancer cells could be used as a therapeutic tool.

B. miRNAs as oncomiRs

OncomiRs regulate cancer-related processes such as cell growth and tissue differentiation. In contrast to the tumor suppressing miRs, O’Donnell et al. found expression of certain miRs that correspond to the miR-17-92 cluster is controlled by overexpressed MYC (O’Donnell et al. 2005). Similarly, miR-21 is established as an oncogenic miRNA and its over-expression has been shown in most cancer types analyzed so far including lung, breast, stomach, prostate, colon, pancreas and cervix (Lui et al. 2007; Frankel et al. 2008; Lu, Z. et al. 2008; Zhu et al. 2008; Li, T. et al. 2009; Xu et al. 2011; Zheng, J. et al. 2011). Apart from these, oncogenic miR-155 has been found to be over-expressed in B-cell lymphomas, breast, lung, colon and
thyroid cancers (Eis et al. 2005; Kluiver et al. 2005; Volinia et al. 2006). These oncomiRs are therefore could be important therapeutic target provided their expression kinetics and oncogenic role is established in a particular cancer like the cancer of cervix.

**Altered miR expressions in cervical cancer**

Recent advances in the field of microRNA resulted in exploration of these small regulators in cervical cancers also. Studies describe a number of microRNAs that are dysregulated in cervical cancer lesions, however, each study demonstrated a unique set of miR that are differentially expressed in cervix cancer (Table LT5) thus making the overall manifestation of miRNA in this disease more complex.

For example miR-21 is overexpressed and expression of miR-143, Let-7c, miR-196b, miR-23b and Let-7b is downregulated in cervix tissue biopsies (Lui et al. 2007). On the other hand Lee et al. demonstrated upregulation of miR-199 and miR-9 and down-regulation of miR-149 and miR-203 in cervical cancer. Similarly, Martinez et al. showed high expression of miR-182 and miR-183 and low expression of miR-126 and miR-143 (Martinez et al. 2008). miR-15b and miR-16 expression was over-expressed in cervical cancer while reduced expression of miR-126 and miR-143 was observed (Wang, X. et al. 2008b). Similarly miR-148a and miR-302b were shown to be overexpressed and miR-203 and miR-513 are downregulated in cervical cancer (Pereira et al. 2010). These study specific variations in miR profile not only reflect population-specific variation but also indicate dynamic nature of miRs in progressive lesions which needs a deeper understanding.

**HPV infection-associated alteration in host miR profile**

Viruses play a major role in regulating host gene expression in many viral infections and many of these responses are mediated through miRs (Roberts and Jopling 2010). In case of HPV infection, expression of viral oncoproteins especially E5, and E6 are specifically linked with a number of miR alterations and the list is expanding with increasing knowledge of miR-based regulation in HPV infection and cervical cancer (Table LT6).
Table LT5: Different microRNA that have been shown to be upregulated or downregulated in cervical cancer

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Expression</th>
<th>Sample type</th>
<th>Technique</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>mir-21</td>
<td>Upregulation</td>
<td>Cervical cancer cell lines and tissues</td>
<td>miRNA cloning</td>
<td>(Lui et al. 2007)</td>
</tr>
<tr>
<td>Let-7b, Let-7c, , mir-23b, mir-143, mir-196b, miR-199-s, miR-9, miR-199a, miR-199b, miR-145, miR-133a, miR-133b, miR-214, and miR-127</td>
<td>Downregulation</td>
<td>Cervical tissues</td>
<td>Taqman real time quantitative PCR</td>
<td>(Lee, J. W. et al. 2008)</td>
</tr>
<tr>
<td>miR-149 and mir-203 miR-182, miR-183 and miR-210</td>
<td>Downregulation</td>
<td>Cervical cancer cell lines</td>
<td>Microarray</td>
<td>(Martinez et al. 2008)</td>
</tr>
<tr>
<td>miR-126, miR-143, miR-145, mir-195 and mir-218</td>
<td>Downregulation</td>
<td>Cervical cancer cell lines</td>
<td>Microarray</td>
<td>(Wang, X. et al. 2008b)</td>
</tr>
<tr>
<td>mir-21, mir-24, mir-27a, and mir-205 mir-143 and mir-145</td>
<td>Downregulation</td>
<td>Cervical cancer cell lines</td>
<td>miRNA cloning</td>
<td>(Wang, X. et al. 2008b)</td>
</tr>
<tr>
<td>miR-15b, miR-16, miR-146a, mir-155 and mir-223</td>
<td>Downregulation</td>
<td>Cervical tissues</td>
<td>Microarray</td>
<td>Hu,2010</td>
</tr>
<tr>
<td>miR-126, and mir-424 miR-200a, miR-9, miR-10b, miR-183, miR-204, miR-24, miR-181a, miR-193b, miR-146b, and miR-10a</td>
<td>Upr egulation</td>
<td>Cervical tissues</td>
<td>Microarray</td>
<td>(Pereira et al. 2010)</td>
</tr>
<tr>
<td>miR-148a, miR-302b, miR-10a, miR-196a, and mir-132</td>
<td>Upr egulation</td>
<td>Cervical tissues</td>
<td>Microarray</td>
<td>(Pereira et al. 2010)</td>
</tr>
<tr>
<td>mir-26a, miR-143, miR-145,mir-99a miR-203, miR-513 miR-29a and mir-199a</td>
<td>Downregulation</td>
<td>Cervical tissues</td>
<td>Microarray</td>
<td>(Pereira et al. 2010)</td>
</tr>
</tbody>
</table>
### Table LT6: HPV- infection induced alteration in host microRNA profile

<table>
<thead>
<tr>
<th>HPV gene</th>
<th>miRNAs</th>
<th>miRNA target</th>
<th>Effect</th>
<th>Sample type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV16 E5</td>
<td>mir-146a↑</td>
<td>↓p38, ERK1/2↑</td>
<td>Suppress differentiation of epithelial cells, attenuated immune response in HPV infections</td>
<td>Cervical cancer</td>
<td>(Greco et al. 2011)</td>
</tr>
<tr>
<td>HPV16 E5</td>
<td>mir-324-5p↓</td>
<td>↑N-cadherin, ↑E-cadherin</td>
<td>HPV E5 oncogene may repress miR-324-5p expression in cervical epithelial cells &amp; contributes to the carcinogenesis</td>
<td>Cervical cancer</td>
<td>(Greco et al. 2011)</td>
</tr>
<tr>
<td>HPV16 E5</td>
<td>mir-203↓</td>
<td>↑p63↑, STAT1↑</td>
<td>E5 acts by suppressing differentiation of epithelial cells through downregulating miR-203 with subsequent upregulation of p63.</td>
<td>Cervical cancer</td>
<td>(Greco et al. 2011)</td>
</tr>
<tr>
<td>HPV16 E6</td>
<td>mir-218↓</td>
<td>↑LAMB-3↑</td>
<td>Downregulation of mir-218 by E6 and overexpression of LAMB-3 may promote viral infection</td>
<td>Cervical cancer</td>
<td>(Martinez et al. 2008)</td>
</tr>
<tr>
<td>HPV16 E6</td>
<td>mir-34a↓</td>
<td>↓p53</td>
<td>Expression of HR-HPV oncoprotein E6 reduces mir-34a expression by destabilizing p53 &amp; promoting cell proliferation</td>
<td>Cervical cancer</td>
<td>(Wang, X. et al. 2008b)</td>
</tr>
<tr>
<td>HPV16 E6</td>
<td>mir-23b↓</td>
<td>↑uPA</td>
<td>p53 mediated the HPV-16 E6 downregulation of miR-23b</td>
<td>Cervical cancer</td>
<td>(Au Yeung et al. 2011)</td>
</tr>
<tr>
<td>HPV E7</td>
<td>mir-203↓</td>
<td>↑p63</td>
<td>E7 blocks mir-203 upregulation Through MAP kinase pathway</td>
<td>Normal Keratinocytes</td>
<td>(Melar-New and Laimins 2010)</td>
</tr>
<tr>
<td>HR HPV</td>
<td>mir-218↓</td>
<td>Not known</td>
<td>mir-218 involvement in pathogenesis</td>
<td>Cervical cancer</td>
<td>(Li, Y. et al. 2010)</td>
</tr>
</tbody>
</table>

E5 was found to induce expression of miR-146a, whereas it repressed miR-324-5p and miR-203. These miR alterations were accompanied by suppressed differentiation and attenuated immune response to HPV infections in cervical epithelial cells (Greco et al. 2011). Similarly, the expression of the HPV16 E6 specifically reduced miR-218 expression, and conversely the expression of epithelial-cell specific marker LAMB3, a target of miR-218, was found upregulated (Martinez et al. 2008). Other HR-HPVs were later described to downregulate miR-218 expression (Li, Y. et al. 2010). Expression of E6 was also found associated with reduction of miR-34a expression in organo-typic tissues derived from HPV-
containing primary human keratinocytes. Reduction of miR-34a expression was attributed to the expression of viral E6, which destabilizes the tumor suppressor p53, a known miR-34a transactivator (Wang, X. et al. 2009b). In addition, E6 oncoprotein decreases the expression of miR-23b that culminates in upregulated expression of its target gene uPA, an inducer of cell migration (Au Yeung et al. 2011). These studies, therefore, indicate to an important role of HPV oncoproteins in alteration of host miRNA profile and this altered miRNA expression profile by itself becomes a major pool of regulators that destabilizes cell growth and survival mechanisms causing events leading to cervical carcinogenesis.

**Strategies targeting miRs in carcinogenesis process**

Accumulating data from the recent studies suggests that miRNAs might be used as potential therapeutic tools for several diseases including cancer. For example, the activity of miRNAs was inhibited using miRNA inhibitors for miR-21 and the cell growth in HeLa was found to increase after miRNA inhibition (Cheng et al. 2005). The reporter vectors containing miRNA binding sites for target miRNAs are constructed. These binding sites are made by hybridizing oligonucleotides containing the miRNA binding site and cloned them into the 3’ UTR of luciferase vector. miRNA inhibitors inhibit the ability of the endogenous miRNA by inhibiting the expression of the reporter gene containing the miRNA binding site (Zeng et al. 2002; Zeng et al. 2003; Hutvagner et al. 2004). Another approach uses 2-O-methyl oligoribonucleotides (2-O-Me-RNA). These molecules stoichiometrically bind and irreversibly inactivate miRNAs. Antisense 2-O-Me-RNA have been used to specifically down-regulate miRNAs in human cells (Hutvagner et al. 2004). Knockdown of miR-21 using 2-O-Me-RNA triggered activation of caspases and increased cell death in glioblastoma cells (Chan, J. A. et al. 2005). Antagomirs are chemically modified cholesterol conjugated single strand RNA molecules complementary to a mature miRNA. miR-122 antagomir administration showed upregulation of genes with 3’UTR miR-122 recognition motifs, leading to a reduction in plasma cholesterol levels (Krutzfeldt et al. 2005). For miRNAs that act as tumour suppressors it may be of interest to develop in-vivo expression systems. For e.g, let-7, a tumor suppressor family, may provide useful strategy to control tumor growth by ectopically overexpressing family (Takamizawa et al. 2004). Locked
nucleic acids (LNAs) comprise a class of bicyclic high-affinity RNA analogues in which the furanose ring in the sugar-phosphate backbone is chemically locked in an RNA-mimicking N-type (C3-endo) conformation by the introduction of a 2-O,4-C methylene bridge (Vester et al. 2002) (see table 3). LNA-ISH (in-situ hybridization) based detection of miR-21 over-expression indicates important diagnostic marker of colorectal carcinogenesis (Yamamichi et al. 2009). The sponge mRNA, which contains multiple target sites complementary to a miRNA of interest, is a dominant negative method (Ebert and Sharp 2010). Down-regulation of miRNA-574-5p using miR-574-5p sponge in-vivo significantly abrogated the enhanced tumor progression induced by TLR9 signaling in human lung cancer (Li, Q. et al. 2012). Expression of constitutively active TORC1 has been shown to attenuate the miR-21 sponge-mediated suppression of proliferation and migration of renal cancer cells (Dey et al. 2012). miRNA target decoys are endogenous RNA that can negatively regulate miRNA activity. An mRNA decoy has been designed and applied in the research of miR-133 in the pathogenesis of cardiac hypertrophy. The suppression of miR-133 decoy sequences induced cardiac hypertrophy (Care et al. 2007). The various strategies to target miRNAs are summarized in Table LT7.

Prognostic significance of miRs in cervical cancer

Despite speculation that progressive cervical or other cancer lesions will have unique set of miR, not much work has been carried out to measure the prognostic significance of miRs in HPV infection or cervical cancer. Altered miR expression has been associated with cancer progression and miR profile as prognostic factor can provide valuable tool in treatment of cervical cancer patient. Recently some studies have shown potential of miRs as prognostic marker in HPV-mediated cancers. Thirty nine cancer-associated miRs were found near 37 HPV integration sites. miR-21, miR-142, miR-301a and miR454 were present at HPV16 integration site at chromosome number 17q23.1 (Nambaru et al. 2009). Among these miRs, miR-21 has been shown to be over-expressed in different cancers. Hu et al. used the recursive feature elimination (RFE) technique to rank the relative importance of each miRNA in cervical cancer samples. Among top 10 miRs, miR-200a and miR-9 were described as promising miRs that could predict patient survival (Hu et al. 2010). Similarly, reduced Let-7 expression is significantly associated with the shortened survival of lung cancer patients (Takamizawa et al. 2004) and may have prognostic value. Based
on these leads, miR signatures have been suggested as novel prognostic indicators which may contribute to the improved selection of patients to classify tumors according to clinico-pathologic variables currently used to predict disease progression. However, these findings need additional experiments to investigate how altered miRNA expression would manifest the biological consequences in the development of cervical cancer.

Table LT7: Various strategies for targeting/overexpressing microRNAs

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Strategy</th>
<th>Characteristics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Locked nucleic acid (LNA)</td>
<td>Unprecendented binding affinity to complementary RNA molecules which is governed by conformational restriction</td>
<td>(Vester et al. 2002)</td>
</tr>
<tr>
<td>2</td>
<td>mRNA decoy</td>
<td>It will be more difficult for diseased cells to evolve resistance to RNA decoy</td>
<td>(Cassiday and Maher 2003)</td>
</tr>
<tr>
<td>3</td>
<td>miRNA Hairpin Inhibitors</td>
<td>RNA oligos with novel secondary structure designed to inhibit the function of endogenous miRNA, chemically enhanced to improve efficacy and longevity</td>
<td>(Meister et al. 2004)</td>
</tr>
<tr>
<td>4</td>
<td>miRNA mimics</td>
<td>Double stranded oligos designed to mimic the functions of endogenous mature miRNA; chemically enhanced to preferentially program RISC with active miRNA strand</td>
<td>(Pillai et al. 2004)</td>
</tr>
<tr>
<td>5</td>
<td>Modified anti-miRNA oligonucleotides (AMOs) or Antagomirs</td>
<td>Effectively silence miRNAs in vivo. Enable the study of gene regulation in vivo by tissue specific miRNAs</td>
<td>(Krutzfeldt et al. 2005)</td>
</tr>
<tr>
<td>6</td>
<td>miRNA inhibitory transgenes or miRNA sponges</td>
<td>Convinience of making dominant negative transgenics over knockouts and applicability to a broader range of model organism and cell lines</td>
<td>(Ebert and Sharp 2010)</td>
</tr>
</tbody>
</table>

miRs as upstream regulators and downstream targets of cellular transcription factors associated with viral transcription

microRNAs as upstream regulators and downstream targets of some of the important transcription factors that control HPV16 URR are presented in Figure LF7 and discussed below.
**p53-** p53 is a transcription factor that binds directly and specifically as a tetramer to target sequences of DNA thereby regulating gene expression (el-Deiry *et al.* 1992; Bourdon *et al.* 1997). p53 induces cell cycle arrest by trans-activating genes such as the cyclin dependent kinase inhibitor, p21 or miR-34. Alternatively, p53 induces apoptosis by transactivating proapoptotic genes such as Bax, Puma, Scotin, and Fas and repressing the anti-apoptotic gene Bcl2 (Lace *et al.* 2009). A highly conserved perfect consensus p53 binding site is located just downstream of the transcription start site in miR-34a promoter (Wang, 2007). Predicted p53 binding site has been also shown in miR-34b/c (He *et al.* 2007). Genes in p53 network e.g. BCL2, SP-1, TP-53) with predicted miR-125b binding sites were found by TargetScan and MicroCosm Target databases in human genome (Le *et al.* 2011). p53 also contains binding site in miR-21 promoter (Fujita *et al.* 2008). miR-106b~25, miR-181a, and miR-32, target P300/CBP-associated factor (PCAF), a p53 positive regulator (Pichierri *et al.* 2008).

**Nuclear factor kappa B (NF-kB)-** The role of NF-kB, a transcriptional regulator, in linking inflammation and tumorigenesis has been supported by accumulating evidences (Karin 2006; Inoue *et al.* 2007). NF-kB is constitutively activated during human cervical cancer progression (Nair *et al.* 2003). HPV16 E6 and E7 proteins modulate the expression and the subcellular localization of NF-kB precursors (Havard *et al.* 2005). Also the HPV16 E5 expression leads to NF-kB activation in part with AP-1 in cervical carcinogenesis (Kim, S. H. *et al.* 2009). Two
putative NF-KB binding sites are reported in the miR-155 promoter (Gatto, Rossi et al. 2008). A positive correlation between miR-155 upregulation and NF-kB activation has been shown by some studies (Rai et al. 2008; Wang, B. et al. 2009a; Xiao et al. 2009; Imaizumi et al. 2010; Bala et al. 2011). NF-kB activation is increased on binding of miR-181b-1 in the promoter regions of STAT3. The increased transcription of miR-181b-1 inhibits CYLD, negative regulator of NF-Kb (Trompouki et al. 2003), production which in turn causes increased NF-kB activation in MCF-10A cells. miR-21 works within the inflammation-transformation positive feedback loop STAT3-mediated regulatory circuits by down-regulating PTEN expression to increase NF-kB activity (Iliopoulos et al. 2010). miR-301 activates NF-kB in a positive feedback loop in which miR-301a represses Nkrf to elevate NF-κB activity and NF-κB promotes the transcription of miR-301a (Lu, Z. et al. 2011) NF-kB binds to a site upstream of the Let-7 RNA coding region. NF-kB activation and subsequent repression of Let-7 result in high levels of IL6. These high levels of IL-6 are required for sufficient binding to the IL6 receptor to cause activation and nuclear entry of the STAT3, which then activates VEGF (Iliopoulos et al. 2009).

Activator protein-1 (AP-1) - AP-1 is a family of transcription factors containing seven members, c-Jun, JunB and JunD in the Jun family and c-Fos, FosB, Fra-1 and Fra-2 in the Fos family. These members can form homodimers or heterodimers within the same family members or with members of other family (Angel and Karin 1991). Two AP-1 binding sites located in the URR of the E6 promoter driving the expression of virus-encoded E6 and E7 oncoproteins have been identified in HPVs (Chan, W. K. et al. 1990; Offord and Beard 1990; Mack and Laimins 1991; Bauknecht et al. 1992; Thierry et al. 1992; Zhao, W. et al. 1997). Mutational inactivation of AP-1 binding sites in the natural context of the HPV16 URR leads to an almost complete loss of the transcriptional activity of the E6/E7 promoter (Butz and Hoppe-Seyler 1993). AP-1 has been shown to develop carcinogenesis in a variety of tissues (Milde-Langosch 2005). Our group demonstrated a significant over-expression of constitutively active AP-1 family members in cervical precancer and cancer tissues (Prusty and Das 2005). Recently miRNA miR-7B has been shown to decrease the translation efficiency of the unstable c-Fos mRNA (Vesely et al. 2009). miR-663 decreases activator protein-1 (AP-1) activity and impairs its upregulation by lipopolysaccharides (LPS) by directly
targeting JunB and JunD transcripts (Tili et al. 2010). TRANSFAC matrices revealed
binding site for AP-1 in miR-21 promoter (Fujita et al. 2008).

“Yin and Yang1” (YY1) - The ubiquitous cellular factor YY1 also known as
UCRBP, δ, NF-E1, CF1, NMP-1, plays a critical role in tumorigenesis (Gordon et al.
2006) and HPV infection (Baritaki et al. 2007) and has been shown to function as
both a positive (Riggs et al. 1993; Satyamoorthy et al. 1993; Mills et al. 1994) and a
negative (Lee, T. C. et al. 1992; Montalvo et al. 1995) regulator of cellular and viral
gene expression. It has been showed that extra-chromosomal or integrated HPV16
DNA isolated from malignant cervical biopsies contains mutated or deleted YY1 sites
upstream of the p97 start site (May et al. 1994; Schmidt et al. 2001) resulting in
increased p97 transcription, origin of replication (ori) function, initial plasmid
amplification and virus immortalization capacity. YY1 binding to a critical motif
adjacent to the p97 transcription start site downregulates the HPV16 E6/E7 promoter
(Lace et al. 2009).” YY1 Regulates miR-190 Expression in the primary hippo-campal
cultures (Zheng, H. et al. 2010).miR-29 is epigenetically silenced by an
activated NF-kB-YY1 pathway in rhabdomyosarcoma cells and primary tumors
(Wang, H. et al. 2008a).

Signal transducers and activators of transcription-3 (STAT-3) -STAT proteins
comprise a family of transcription factors which are latent in the cytoplasm and when
activated they participate in normal cellular events, such as differentiation,
proliferation, cell survival, apoptosis, and angiogenesis following cytokine, growth
factor, and hormone signaling (Calo et al. 2003). Over-expression of STAT3, one of
the important member of the STAT family, has been observed in a wide number of
human cancer cell lines and primary tumors including blood malignancies, solid
neoplasias(Buettner et al. 2002) and cervical cancer (Shukla et al. 2009a). Current
literature indicates a strong interaction of STAT3 signaling with HPV infection during
cervical carcinogenesis. STAT3 activation may serve as an important player in HPV-
mediated cell cycle dysregulation. UpregulatedSTAT3 expression is expected to
repress the de-novo production of p53 whereas, E6 mediate the degradation of
already produced p53 proteins thereby critically depleting the cellular p53. STAT3
binding site has been shown in the promoters of different miRs including
miR-21(Loffler et al. 2007; Fujita et al. 2008) and miR-148a, miR-21, miR-132,
miR-181b-1, miR-148b, miR-193a, miR-340, miR-335, miR-210 and miR-187 were identified (Iliopoulos et al. 2010). 130-bp regions containing two predicted STAT3 binding sites upstream of the miR-21 genes have been reported in various vertebrate species. A stable distance between miR-21 and the STAT3 sites throughout all vertebrates strongly suggests their functional correlation (Löffler et al. 2007). Using computational based PhylCRM score putative STAT3 binding sites in the promoters of miR-148a, miR-21, miR-132, miR-181b-1, miR-148b, miR-193a, miR-340, miR-335, miR-210 and miR-187 were identified. miR-181b-1 is found to be trans-activated by STAT3. STAT3 and miR-181b-1 expression levels are positively correlated in colon adeno-carcinomas as well as in MCF-10A cells during transformation (Iliopoulos et al. 2010). miR-20b reduces VEGF expression through HIF-1 and STAT3 mediation in breast cancer cells (Cascio et al. 2010). miR-21 negatively regulates PTEN, negative regulator of STAT-3 and MMP-2, expression up-regulating MMP-2 expression in cardiac fibroblasts (Roy et al. 2009). SOCS-1 has been implicated in the negative regulation of IL-6R/Jak/STAT pathways (Greenhalgh and Hilton 2001). miR-19a and miR-19b has been shown to target SOCS-1 and suggest a role of miR-19s in the IL-6 anti-apoptotic signal in the pathogenesis and malignant growth of multiple myeloma (Pichiorri et al. 2008).

Approximately 50 miRNAs are predicted to bind the 3’-UTR of STAT3, of which Let-7, miR-20a and miR-93 were directly validated using STAT3-3’-UTR-Reporter constructs (Foshay and Gallicano 2009; Wang, Y. et al. 2010). Regulatory circuits involving STAT3 and miRNAs play important roles during stem cell differentiation and early organogenesis (Haghikia 2012). Further characterization of Let-7a in hepatoma cells implied that it negatively regulates cellular proliferation through direct targeting of STAT3 (Wang, Y. et al. 2010). STAT3 mediated processing of individual miRNAs and contrariwise, the modulation of the STAT3 pathway by miRNAs at different levels is shown in Fig. LF8.
Figure LF8- Scheme illustrating STAT3 mediated processing of individual miRNAs and contrarywise, the modulation of the STAT3 pathway by miRNAs at different levels. STAT3 mediates positive and negative regulation of various miRNAs at the transcriptional level. On the other hand, this signaling pathway is controlled by numerous miRNAs at the receptor level, by modulating its activators and suppressors and by direct regulation of STAT3 mRNA (Haghikia 2012).

STAT3 mediates positive and negative regulation of various miRNAs at the transcriptional level. On the other hand, this signaling pathway is controlled by numerous miRNAs at the receptor level, by modulating its activators and suppressors and by direct regulation of STAT3 mRNA (Haghikia 2012). Some of the important components of STAT3 signaling as direct targets of microRNAs (Fig. LF9A) and as downstream targets of microRNAs are shown in Fig. LF9B.
Figure LF9– Important components of STAT3 signaling as (A) direct targets of microRNAs and (B) as downstream targets of microRNAs.

**microRNA-21 and cancer**

microRNA-21 also known as hsa-miR-21 or miRNA-21 is a mammalian microRNA that is encoded by the miR-21 gene. The human microRNA-21 gene is located on plus strand of chromosome 17q23.2 (55273409–55273480) within a coding gene TMEM49 (also called vacuole membrane protein; (Fig. LF10). It has its own promoter regions and forms a ~3433-nt long primary transcript of miR-21 (known as pri-miR-21) which is independently transcribed. The stem–loop precursor of miR-21(pre-miR-21) resides between nucleotides 2445 and 2516 of pri-miR-21.

miR-21 is one of the first microRNA to be described as an oncomiR. miR-21 is frequently up-regulated in cancer and the majority of its reported targets are tumor suppressors. miR-21 suppresses the expression a large number of genes that participate directly or indirectly in the extrinsic or intrinsic apoptosis pathways to promote tumorigenesis.
miR-21 is found to be upregulated in several cancers including breast, colorectal, ovarian, hepatocellular, cervical, pancreatic cancer, chronic lymphocytic leukemia, uterine leiomyoma, cholangiocarcinoma, glioblastoma and lung cancer (Chan, J. A. et al. 2005; Meng et al. 2006). It is found to be downregulated in pituitary adenomas (Chan, J. A. et al. 2005; Lee, Y. S. and Dutta 2009). The qPCR expression profile of miR-21 in different cancer has been shown in Fig. LF11.

Scores of miR-21 target genes have been identified and their functions in cancer have been revealed. Interestingly, these targets have been found to play a key role in virtually every one of the ten biological capabilities acquired or needed for tumor development. However, the vast majority of studies using cell lines, and two transgenic and knockout mouse models of miR-21, support the notion that miR-21 exerts its oncogenic function predominantly through the inhibition of cellular apoptosis (Fig. LF12).
miR-21 is a negative regulator of p53 signaling and NF-κB signaling is promoted by miR-21. Inactivation of p53 and NF-κB activation are implicated in deregulation of glucose flux and oxidative phosphorylation. It is notable that several targets contribute to more than one hallmark, and that virtually every hallmark involves at least one miR-21 target (Buscaglia and Li 2012).

This extensive miRNA profiling in different cancers compared with normal tissues/cells identified miR-21 as a key molecule associated with neoplastic transformation and as a biomarker (Mudduluru et al. 2010). miR-21 is an oncogene which plays a key role in resisting programmed cell death in cancer cells and that targeting apoptosis is a viable therapeutic option against cancers expressing miR-21 (Buscaglia and Li 2012). Recent evidence suggests that miR-21 promotes glioma (Gabriely et al. 2008) and cholangio-carcinoma (Selaru et al. 2009) invasion by targeting MMP regulators. Tissue inhibitors of metalloproteinases (TIMPs) contain a consensus miR-21 binding site (Long et al. 2009) (Long, Sun et al. 2009) (http://targetscan.org/; (Longworth and Laimins 2004) (Longworth and Laimins 2004) http://pictar.mdc-berlin.de http://microRNA.org), and reduced expression of TIMP3 in breast cancer tissue has been associated with poor disease-free survival (Mylona et al. 2006). It has been suggested that miR-21 could promote invasion in breast cancer cells via its regulation of TIMP3 (Song, B. et al. 2009).
microRNA Let-7a and cancer

miRs might serve as good targets or candidates in an exploration of anticancer therapeutics. One attractive candidate for this purpose is Let-7 (“lethal-7”). Let-7miRNA, the second member of the miRNA family discovered in 2000 (Jeong et al. 2011). Let-7 is under-expressed in various cancers, and restoration of its normal expression is found to inhibit cancer growth by targeting various oncogenes and inhibiting key regulators of several mitogenic pathways. However, the exact role of Let-7 in cancer is not yet fully understood. Let-7 acts as a tumour suppressor by targeting various oncogenes and key components of the cell cycle and developmental pathways. Most reports reveal that Let-7 is frequently under-expressed and that the chromosomal region of human Let-7 is frequently deleted in many cancers (Calin et al. 2004). Similarly, in more differentiated tumour cells, Let-7 is expressed at higher levels, and its target oncogenes (HMGA2 and ras) are down-regulated. Thus, loss of Let-7 expression is a marker for less differentiated cancer (Shell et al. 2007). Let-7 was first discovered and well studied in Caenorhabditis elegans, in which it regulates developmental timing (Reinhart et al. 2000; Grishok et al. 2001; Abbott et al. 2005). In humans, 12 genomic loci encode the Let-7 family members (Let-7a-1, -2, -3; Let-7b; Let-7c; Let-7d; Let-7e; Let-7f-1, -2; Let-7g; Let-7i; miR-98). Human Let-7 is upregulated during embryonic cell differentiation. The biogenesis of Let-7 is similar to that of other miRNAs (Barh et al. 2010). Let-7 regulates many transcription factors that play important roles in regulation of the cell cycle, cell differentiation, and apoptosis. Many of the factors controlling the expression of Let-7 form regulatory circuits with the factors being regulated by such expression.

The, Let-7 gene family consists of 11 very closely related genes and Let-7a is currently the best characterized member. Let-7a is abundant in differentiated adult tissues and inappropriate expression of Let-7a may result in oncogenic loss of differentiation (Pasquinelli et al. 2000; Thomson et al. 2004). Let-7a was found to act as a tumor suppressor directly regulating RAS and HMGA2 oncogenes by interacting with the 3’UTR (Johnson et al. 2005; Lee, Y. S. and Dutta 2007; Qian et al. 2009). Let-7a is involved in cell proliferation and influences cancer metastasis in various tumors, including breast cancer. Evidence collected to date shows Let-7a was linked to the modulation of different target genes, the most well-known being the RAS family (Yang, Q. et al. 2011). Levels of Let-7a miRNA
reflect the expression of three mature miRNAs: Let-7a-1, Let-7a-2, and Let-7a-3 (Lu, L. et al. 2007). To date, the expression of Let-7a miRNA has been considered to be decreased in cancer cells (Jeong et al. 2011).

**Lacunae in STAT3/miRs correlation in cervical cancer**

Although STAT3 over-expression has been reported in several cancers but its aberrant regulation has not been clearly understood. So the present study has been designed with a focus on involvement of two microRNAs- miR-21 and Let-7a which are reported to be over-expressed and down-regulated respectively in different human cancers. We focused on these two microRNAs since their involvement may be responsible for maintaining the aberrant activation of STAT3 in cervical carcinogenesis. Also the miRs aberrant expression has been reported to contribute carcinogenesis but how does this aberrant expression of miRs co-ordinate with transcription factors such as STAT3 remains unclear. So it is quite useful in regard of these lacunae to explore the miR based investigation in regulation of STAT3 to reveal the hidden views in the progression of cervical carcinogenesis.