Chapter 1

General Introduction
A. Genetic Basis of Viral Carcinogenesis

1.1 Tumor Causing viruses

Human body hosts various infectious agents since their evolution from the primate race. Any infection is generally disadvantageous to the host and strongly select for host anti-infectious mechanisms. Therefore, the pathogens must develop counter-mechanisms to maximize reproductive fitness within the human host [Marques et al., 2007]. This arms race between pathogen and host reach a state of equilibrium where the host is not greatly disadvantaged in its reproductive capacity by the infection, and the pathogen is not too limited in reproductive capacity by the host immune response. Yet only a small fraction of such infections becomes fatal to the individual host by engendering tumorigenesis. According to World Health Organization (WHO), approximately 20% of the global cancer burden is associated to chronic infections that include the bacterium, Helicobacter pylori, the parasites particularly Schistosoma haematobium, Opisthorchis viverinni and Clonorchis sinensis. But more than 15% of human cancer is characterized by DNA or RNA viral etiology [zur Hausen H, 2006].

Figure 1.1: Annual global cancer incidence due to infection [modified from: Parkin DM et al., 2002]
They include two members of the Herpes virus family, Epstein-Barr Virus (EBV) and Human Herpes Virus Type 8 (HHV-8), High Risk and Low Risk Human Papillomaviruses (HPV), Hepatitis B and C Viruses (HBV & HCV), Human Polyomavirus, Merkel cell polyomavirus (MCPyV), the Human T-Lymphotropic retrovirus type 1 (HTLV-1), and Human Immunodeficiency Viruses types 1 and 2 (HIV-1, 2) [zur Hausen H, 2006, Feng H et al., 2008]. In addition, human endogenous retroviruses have been suspected to play a role in human cancers. An estimate of the present contribution of infectious agents to global cancer incidence is shown in Figure 1.1.

As the viral prevalence in host population varies with geographical regions, ethnicity, socioeconomic status, cultural practices, habits and lifestyle the virus associated cancer incidence shows diversity in its global distribution and etiology. In 1990, Evans and Mueller proposed epidemiologic guidelines to support the etiologic role of a virus as cancer causative agent (Table 1.1).

Table 1.1: Evans and Mueller guidelines to support the etiologic role of a virus as cancer causative agent [Ref. Evans AS et al., 1990].

| 1. Geographic distribution of viral infection should coincide with that of the tumor, adjusting for the presence of known cofactors. |
| 2. Presence of viral markers should be higher in case subjects than in matched control subjects. |
| 3. Viral markers should precede the tumor, with a higher incidence of tumors in persons with markers than those without. |
| 4. Prevention of viral infection should decrease tumor incidence. |

Suggested epidemiologic guidelines:
1. Virus should be able to transform human cells *in vitro*.
2. Viral genome should be demonstrated in tumor cells, not in normal cells.
3. Virus should be able to induce the tumor in an experimental animal.
1.2 RNA & DNA Viruses: Mode of Carcinogenesis

The first evidence of tumor viral etiology dates back to 1907 when Ciuffò and co-workers showed that human warts could be transmitted by cell-free filtrates derived from the lesions [Ciuffò G. Giorn Ital Mal. Venereol, 1907]. Later on, in 1911, Rous et al. showed that the spindle cell sarcoma could be transmitted to healthy chickens using filtered cell-free tumor extracts [Rous P. J Exp Med, 1911]. This study led to the identification of the first oncogenic virus: the Rous sarcoma virus (RSV). Over the next few decades after the discovery of RSV new tumor viruses were identified. The discovery of new tumor viruses got a new dimension in 1974 when Harald zur Hausen had proposed for the first time that the Human Papillomavirus (HPV) may represent the etiologic agent for cervical cancer [zur Hausen H et al., 1974, zur Hausen H, 1976].

Based on the mechanisms involved in the carcinogenesis, the viruses are often classified into Direct Carcinogens and Indirect Carcinogens [zur Hausen, 2006].

- **Direct Carcinogens:** When the expression of some specific viral Oncogenes are obligatory for maintenance of the malignant phenotype of the tumor cells. E.g. HPV, EBV, HHV, HTLV-1 and MCPyV, etc.

- **Indirect Carcinogens:** Some viruses are known as indirect carcinogens as persistence of their genes within the cancer cells are not mandatory for maintenance of the malignant phenotype. E.g. HIV-1, 2; HBV, HCV, cutaneous HPVs, etc.

On the basis of the nature of nucleic acid present as genomic material in the human oncogenic viruses, they are broadly classified into: Oncogenic RNA and DNA viruses.

1.2.1 Oncogenic RNA viruses: The RNA viruses associated with human cancer are mainly included in Retroviridae and Flaviviridae families [Bergonzini et al., 2010]. Among
them HCV, HTLV-1 and HIV are the established carcinogenic members. They usually characterized by the ability to carry or alter important cellular growth-regulatory genes, the oncogenes. The proteins encoded by these cellular genes are not essential for viral replication and are usually key player in cell cycle control. The viral oncoproteins from the three retroviruses and their cellular targets have been tabulated in Table 1.2.

Table 1.2: List of cellular and viral protein interactions involved in RNA virus-related oncogenic transformation [Ref. Bergonzini et al., 2010]

<table>
<thead>
<tr>
<th>RNA Virus</th>
<th>Viral oncoprotein</th>
<th>Cellular targets</th>
<th>Deregulated signaling pathway</th>
<th>Tumor types</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTLV-1</td>
<td>Tax</td>
<td>NFκB, Akt, PDZ protein p300/CBPp53 CREB 2</td>
<td>Cell-cycle, Apoptosis, Cellular transcription, NFκB, PI3K/AKT, Chromatin remodeling, T-cell activation cascade</td>
<td>Adult T cell Leukemia/Lymphoma (ATL)</td>
</tr>
<tr>
<td></td>
<td>HBZ</td>
<td>MHC-I, STAT-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1</td>
<td>Tat</td>
<td>pRb2/p130</td>
<td>Cell-cycle, Apoptosis, Cellular transcription</td>
<td>Kaposi Sarcoma (KS), Non-Hodgkin's Lymphomas (NHL), Uterine Cervical Carcinoma (CaCx)</td>
</tr>
<tr>
<td>HCV</td>
<td>Core and non-structural proteins (NS5A)</td>
<td>hTERT, p53, Rb</td>
<td>Cell-cycle, Apoptosis, Ras-Erk MAPK pathway, PI3K, NFκB</td>
<td>Hepatocellular Carcinoma (HCC)</td>
</tr>
</tbody>
</table>

1.2.2 **Oncogenic DNA viruses:** Compared to the RNA viruses the DNA tumor viruses are ubiquitous (example includes the Herpesvirus, Polyomavirus, Adenovirus, and Papillomavirus family members), causing cell transformation by encoding proteins of exclusively viral origin and essential for viral replication [Saha A et al., 2010]. The oncogenes they carry target cellular tumor suppressor proteins such as p53 and pRb gene product. Most of these viruses integrate into the host genome and have the ability
to immortalize the target cell to allow their own replication. The infected cell expresses the viral genes, which induces cell growth, proliferation and prevent apoptosis. The viral oncoproteins from the five most potent DNA oncoviruses and their cellular targets have been tabulated in Table 1.3.

**Table 1.3: List of cellular and viral protein interactions involved in DNA virus-related oncogenic transformation [Ref. Saha A et al., 2010].**

<table>
<thead>
<tr>
<th>DNA Virus</th>
<th>Viral oncoprotein</th>
<th>Cellular targets</th>
<th>Deregulated signaling pathway</th>
<th>Tumor types</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPVs</td>
<td>E6</td>
<td>p53, p73, E6AP, CBP/p300, c-Myc</td>
<td>Cell-cycle, DNA-repair, Apoptosis, Ub-proteasome,</td>
<td>Uterine Cervical Carcinoma (CaCx), Anogenital Cancer, Vulvar Cancer, Penile Cancer, Head &amp; Neck Squamous Cell Carcinoma (HNSCC), Breast carcinoma (CaBr)</td>
</tr>
<tr>
<td></td>
<td>E7</td>
<td>pRb, pRb pocket proteins, p21, p27, IRF-1, Cyclin A and E</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E5</td>
<td>EGFR, p21, p27, ETAR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV</td>
<td>EBNA2, 3C</td>
<td>RBP-Jκ, PU.1, AUF1, DDX20, SMN, p53, Mdm2, pRb, Chk2, c-Myc, HDAC1, SUMO-1, SUMO-3</td>
<td>Cell-cycle, Notch, Ub-proteasome, Chromatin remodeling, Cellular transcription, Apoptosis, Inflammation</td>
<td>Burkit’s Lymphoma (BL), Naso-pharyngeal Carcinoma (NPC), Non-Hodgkin's Lymphomas (NHL)</td>
</tr>
<tr>
<td>LMP1, 2</td>
<td>TRAFs 1, 2, 3 and 5, TRADD, RAS, JAK, TNFR associated factors, RAS, JAK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV</td>
<td>HBx</td>
<td>NFκB, p53, c-jun, c-fos, PKC, c-myc, SP1, HIF-1α</td>
<td>Cell-cycle, Apoptosis, cellular transcription, NFκB Wnt/β-catenin, TGFβ, JAK/STAT</td>
<td>Hepatocellular Carcinoma (HCC)</td>
</tr>
<tr>
<td>MCPyV</td>
<td>LT</td>
<td>p53, pRb</td>
<td>Cell cycle, PKC, Inflammation</td>
<td>Merkel cell carcinoma (MCC)</td>
</tr>
<tr>
<td></td>
<td>NS3</td>
<td>p53, Arginine methyltransferase 1, PKA, H2B, H4</td>
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</table>
1.2.3 Mode of Viral Carcinogenesis

The complexity of cellular deregulation induced by the expression of viral-oncoproteins and involvement of various signaling pathways in cancer development is depicted in Figure 1.2.

Figure 1.2: An overview on viral mode of carcinogenesis [adapted and modified from Saha A et al., 2010].
The oncogenic viruses induce cellular deregulation by the expression of viral oncoproteins. These oncoproteins modulate numerous signaling pathways that lead to immortalization of the infected cell through deregulation of cellular homeostasis, promote an aberrant cell-proliferation and escape from cellular defense system such as blocking apoptosis. Subsequently, primary cancerous cells become metastatic through inhibiting cellular metastasis suppressor proteins.

Referred to the Table 1.2, 1.3 and Figure 1.3 the malignant phenotype of the viral infected cells is chiefly achieved by targeting the key cellular proteins. By inducing p53 degradation or abrogation of its stability in cell the DNA-repair, cell-cycle control, apoptotic pathways are deregulated. Similarly, by degradation of Rb or disrupting the negative interaction of Rb with E2F, the viral oncoproteins drive the infected cells to uncontrolled proliferation. Down regulation of the negative regulators of cell cycle, viz. p21 and p27, further induces abnormal cell proliferation.

![Figure 1.3: Deregulation of key cellular proteins (in black) involved in DNA repair, cell-cycle and apoptotic pathways by the oncoproteins (in red) encoded from various human tumor viruses [adapted and modified from Saha A et al., 2010].](image-url)
1.3  *Host-immune response against viral infection*

The viral oncoproteins are needed to persist within the infected cells to destabilize its normal homeostasis. But, due to its extrinsic nature, the oncoproteins invariably face strong defense from the host immune system. Upon stable infection of the oncoviruses, the adaptive immune system of the host comes into play for successful immune-clearance of the virus. The mechanism has been illustrated in the following Figure 1.4.

![Figure 1.4: Host cellular-immune response against onco-viral infection [adapted from Tindle RW, 2002]](image)

At first, the Dendritic cells (DC) collect the viral onco-proteins from the infected cells and are processed into peptides. Eventually, the DCs mature in the presence of inflammatory signals supplied by the Cytokines (eg. Interleukins, Interferons) due to wound, tumor necrosis factors (TNF) or other signals [Tindle RW, 2002]. Subsequently, they acquire co-stimulatory molecules belonging to the B7 (CD80/86)
superfamily on their surface. These mature DCs then migrate to the regional lymph nodes where processed peptides bound to Major Histocompatibility Complex class I or class II molecules (MHC I/II) on DCs [Tindle RW, 2002]. The MHC antigen bound viral peptides were then cross presented to CD8+ and CD4+ T-cells, respectively, which bear specific T-cell receptors (TCRs). Concomitantly, an activation stimulus is generated to transform these T-cells to effector cells, viz. CD8+ Cytotoxic T-cells and CD4+ helper T-cells [Tindle RW, 2002]. The effector cells then migrate to the tumor tissues and particularly attack the cells bearing tumor-antigens. The Cytotoxic T-cells kills the viral infected cells while the helper cells supplement cytokines for the immune-response. Furthermore, to promote the immune-response, Antigen Presenting Cells (APC) is engaged to secrete mediators to enhance the inflammatory reaction [Tindle RW, 2002].

1.4 *Oncogenic viral strategies for host immune-evasion*

According to Iannello et al., [Iannello A et al., 2006] the human onco-viruses may undertake the following steps in order to go against the host-immune mechanisms of viral clearance:

*Processing of viral peptides:*

- Blocking the transporter associated with antigen processing (TAP) function and the transport of viral peptides into Endoplasmic Reticulum (ER).
- Inhibiting proteasomal degradation of the viral protein.

*Interference with MHC Class I & II function:*

- Decreasing the transcription of MHC class I & II genes.
- Enhanced ubiquitinylation and degradation of the MHC antigens.
- Inhibiting intracellular transport of MHC class I heavy chains.
- Interfering with viral peptide loading to altered structure of the antigen binding domain of MHC II, due to the presence of Single Nucleotide Polymorphisms (SNP) in the coding sequence.
- Interfering with TCR-MHC class II interactions.
Additionally, the viruses evade host’s cytokine and chemokine responses by encoding:

- Viral versions of cytokines and chemokines.
- Cytokine and chemokine receptor homologues.
- Cytokine and chemokine binding proteins.
- Viral proteins with chemokine-like activity.

1.5 Inflammatory response and viral carcinogenesis: the enemy within

Generation of inflammatory response, specifically chronic inflammation, is one of the important host immune mechanisms to eliminate viral infection [Coussens LM et al., 2002]. Inflammatory cytokines of IL-1 family (IL-1α, IL-1β and IL-1 Receptor antagonist, IL-1Ra), produced by the host monocytes and tissue-macrophages, are the key regulators of inflammatory response [Bird S et. al, 2002; Dinarello CA, Blood, 1996]. Among them, IL-1α and IL-1β, besides causing inflammation also induce expression of other pro-inflammatory genes viz. IL-2, cyclooxygenase type 2, IL-6, inducible nitric oxide synthase, TNFα and other cytokines/chemokines [Dinarello CA, Blood, 1996]. These pro-inflammatory cytokines may in turn enhance carcinogenesis by inducing growth factors for pre-malignant cells and producing oxidative stress, mutagenic to the onco-viral infected cells [Dinarello CA, Blood, 1996]. For this reason altered expression of these pro-inflammatory cytokines are associated in various human tumor types including virus associated cancers.

Several studies have reported the association of altered expression of pro-inflammatory cytokines, such as in IL-1β, IL-6, IL-10 etc. with presence of genetic polymorphism in their genes [Al-Tahhan MA et al., 2011; Liu J et al., 2006; El-Omar EM et al., 2000; Lee KA et al., 2004; Zienolddiny S et al., 2004; Hirankarn N et al., 2006; Shi TH et al., 2013; Barbisan G et al., 2012; Chakravorty M et al., 2006]. Most of these single nucleotide polymorphisms (SNP) are localized in the coding regions or in the upstream promoter region and exert positive/negative impact on expression of
these genes. Thus, occurrence of such polymorphisms and their allelic composition 
predisposes the individual to viral carcinogenesis.

B. Uterine Cervical Carcinogenesis: Role of High Risk Human 
Papillomavirus (HR HPV)

1.6 HR HPVs: Prevalence in population

1.6.1 A brief know how about HPV

The Human Papillomavirus (HPV) is one of the most common Papovaviridae families 
of viruses in today’s world [de Villiers et al., 2004]. Because of its epitheliotropic 
nature, HPV infects cells inside and outside of the body and causes ‘Papilloma’- a 
benign growth on the skin or mucous membrane. Such papillomas are seen on surfaces 
of the skin, lining of the mouth, tongue, throat, tonsils, vagina, penis, cervix and anus. 
HPV is also the world’s most common sexually transmitted infection and is 
transmitted by sexual including genital skin-to-skin contact. The ethnicity and socio-
cultural practices including individuals’ age, number of sexual partners, age at sexual 
debut, age at first child birth, parity, use of oral contraceptives, tobacco habit etc. are 
the co-etiological factors for HPV infection [Cotton SC et al., 2007].

HPV is a non-enveloped DNA virus with icosahedral symmetry formed by 72 
capsomeres (Figure 1.5 A) [Doorbar J, 2005]. Its genome contains a single molecule of 
dsDNA, ~7.9 Kb length, which is functionally subdivided into three regions: early, 
late and the regulatory-Long Control Region (LCR) or Non-coding Region (NCR) 
(Figure 1.5 B) [Doorbar J, 2005].

- **The Early region** encompasses over 50% of papillomaviral genome, contains 
six common open reading frames (ORF) viz. E1, E2, E4, E5, E6, E7 [Doorbar 
J, 2005].
- **The Late region** is localized downstream to the early ORFs, encompasses nearly 40% of papillomaviral genome. The late region contains the ORFs for viral major (L1) and minor (L2) capsid proteins [Doorbar J, 2005].

![Diagram](image_url)

Figure 1.5: Diagrammatic representation of three-dimensional structure of Human Papillomavirus type 16 (A), its genomic organization (B) and mRNAs transcribed from the early (P97) and late (P670) promoters (C), respectively [Courtesy Doorbar J, 2005; Schwartz S, 2008].

- **The Long Control Region (LCR)** is ~850 bp long (nt7445-153) non-coding segment of the Papillomavirus genome with crucial regulatory function. This region has the origin of replication, multiple binding sites for various cellular transcription factors (Sp1, AP1, NF1, YY1, Oct-1, C/EBP etc.) and four binding sites (consensus palindromic sequence 5’-ACCGN4CGGT-3’) of its own E2 protein (E2BS1-4) [Dong et al. 1994; May et al. 1994; Park et al.].
Table 1.4: The Human Papillomavirus (HPV) proteins and their functions.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E1</strong></td>
<td>A prerequisite for extrachromosomal viral DNA replication. It has DNA-binding functions and a binding site in the origin of replication (OriC) localized in the long control region (LCR).</td>
</tr>
<tr>
<td><strong>E2</strong></td>
<td>The gene consist of N-terminal transactivation domain, hinge region and C-terminal DNA binding domain. The viral genome gets integrated into the host genome by disrupting this hinge region. The E2 proteins regulate transcription of the viral early genes by binding to the E2 binding sites (E2BS) located in LCR. Also control DNA replication and segregation of viral genomes.</td>
</tr>
<tr>
<td><strong>E4</strong></td>
<td>Expressed in later stages of viral replication. It favors and supports the HPV genome amplification, regulates the expression of late genes, controlling the virus maturation and facilitates the release of virions.</td>
</tr>
<tr>
<td><strong>E5</strong></td>
<td>Enhances the transforming activity of E6 and E7. Interacts with cell membrane receptors, such as EGFR (epidermal growth factor receptor), PDGFR (platelet derived growth factor receptor) and G protein-coupled endothelin receptor (ETA)/ET1 and may stimulate proliferation of infected cells, promotes fusion between cells and also induces evasion of immune response.</td>
</tr>
<tr>
<td><strong>E6</strong></td>
<td>Utmost important of viral replication, immortalization and transformation of infected cells. Binds to the tumor-suppressor protein p53 and stimulates its degradation through ubiquitination. Also inhibits apoptosis by binding to Bak, Bax etc. Interacts with proteins of the innate immune response; activates the expression of telomerase.</td>
</tr>
<tr>
<td><strong>E7</strong></td>
<td>Utmost important of viral replication, immortalization and transformation of infected cells. Binds and degrades the tumor-suppressor protein pRB; increases CDK activity; affects the expression of S-phase genes by directly interacting with E2F factors and with histone deacetylases; induces a peripheral tolerance in Cytotoxic T-lymphocytes; downregulates the expression of TLR9, contributing to evasion of immune response.</td>
</tr>
<tr>
<td><strong>L1</strong></td>
<td>It is the 56-60 KD, weekly phosphorylated major capsid protein with high self assembly capacity but without having DNA-binding property. It contains the major determinant required for attachment to cell-surface receptors. L1 is highly immunogenic and has conformational epitopes that induce the production of neutralizing type-specific antibodies against the virus.</td>
</tr>
<tr>
<td><strong>L2</strong></td>
<td>It is the 49-60KD, highly phosphorylated minor capsid protein with high DNA binding property and weak self-assembly capacity; L2 contributes to the binding of virion in the cell receptor, favoring its uptake, transport to the nucleus, and delivery of viral DNA to replication centers. L2 also helps the packaging of viral DNA into capsids.</td>
</tr>
</tbody>
</table>
• 1999; Dell and Gaston 2001]. The enhancer (nt7535-7862) region located
within the LCR, also controls the expression of E6/E7 transcript [Baker and
Calef 1995; Dell and Gaston 2001; Doorbar 2006; Smotkin 1989; Tang 2006].
Thus, this region is of utmost importance for RNA polymerase-II mediated
transcription of early and late ORFs [Doorbar J, 2005].

The detailed functions of different early and late proteins are tabulated in Table 1.4.

1.6.2 Classification of HPVs

Figure 1.6: Phylogenetic classification of Papillomaviruses [adapted from de Villiers et
al., 2004]

On the basis of nucleotide sequence homology, evolutionary phylogeny and tropism,
more than 118 different types of Papillomaviruses (PVs) have been identified [de
Villiers et al., 2004]. The phylogenetic classification of Papillomaviruses has been
diagrammed in Figure 1.6. The L1 open reading frame (ORF) is the most conserved gene within the viral genome and has been used for identification of new Papillomavirus types. On the basis of L1 ORF sequence homology, Papillomaviruses have been further grouped into Alpha, Beta, Gamma, Delta, Epsilon, Zeta, Eta, Theta, Lota, Kappa, Lambda, Mu, Nu, Xi, Omicron and Pi genuses [de Villiers et al., 2004]. Each genus may comprise of several species of close phylogeny. Within each species, Papillomavirus types are recognized by more than 10% difference in L1 ORF sequence from the closest Papillomavirus type. Furthermore, differences between 2% and 10% homology define a subtype and less than 2%, a variant [de Villiers et al. 2004]. Among these, only Alpha, Beta, Gamma, Mu and Nu-papillomaviruses infects human. Alpha-papillomaviruses such as HPV type 16, 18, 33, 45 etc causes Cervical Cancer (CaCx), Oral cancer. While HPV type 6, 11, 13 etc causes anogenital warts [Doorbar J, 2005]. HPVs of other genus such as Beta, Gamma, Mu and Nu-papillomaviruses causes skin warts, Cutaneous Papilloma or Non Melanoma Skin Cancer (NMSC) [Doorbar J, 2005].

Table 1.6: Classification of HPV types on oncogenecity (Ref. Munoz et al., 2003).

<table>
<thead>
<tr>
<th>Risk Association</th>
<th>HPV types</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Risk</td>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82</td>
</tr>
<tr>
<td>Probable High Risk</td>
<td>26, 53, 66</td>
</tr>
<tr>
<td>Low Risk</td>
<td>6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81</td>
</tr>
<tr>
<td>Undetermined risk</td>
<td>34, 57, 83</td>
</tr>
</tbody>
</table>

Based on association with CaCx, precancerous lesions and oncogenicity, Munoz et al. classified HPVs into High Risk (HPV 16, 18, 31, 33, 45, 51, 56 etc), Probable High Risk (HPV 26, 53, 66), Low Risk (HPV 6, 11, 40, 42, 54, 61 etc) and Undetermined Risk (HPV 34, 57, 83) types (Table 1.5) [Munoz et al., 2003].

1.6.3 Global scenario of HR HPV prevalence in uterine cervix of normal women

Genital infection of HPV is very common in human population and because of the tissue architecture the mucosal epithelium of uterine cervix harbors a maximum
prevalence of HPV than the rest of the anogenital regions. The estimated worldwide prevalence of HPV infection in cervix is ~10%, as observed in different cross-sectional studies [Clifford GM et al., 2005; de Sanjose S et al., 2007; Bruni L et al., 2010; Crow JM, 2012]. The global prevalence of HPV infection in women with normal cervical cytology has been shown in Figure 1.7.

According to Crow JM, 2012, the Sub-Saharan Africa shares the maximum HPV burden around the globe (22.9%) followed by Central America & Mexico (20.5%), whereas, the prevalence is lowest in the European countries (6.6%) (Figure1.7). The prevalence varies from 8.3% to 14.3% in Asia, North America and South American continents. Thus, the HPV prevalence is higher in the under developed countries than the developing and developed countries in the world.

Among the HPV types prevalent in the cervix around the world, the most common were HPV16, 18, 52, 31, 58, 39, 53, 56, 33, 45 (Figure 1.8 A) [Bruni et al., 2010; Crow JM, 2012]. Interestingly, all these prevalent types belong to oncogenic High Risk group, although, the type prevalence varies from continent to continent (Figure 1.7). This is possibly due to diverse demography and ethnicity of human population throughout the world. About 22.5% of the global HPV infection is estimated to be produced by HPV16. In the Sub-Saharan Africa, HPV16 having lowest contribution to the overall HPV prevalence (13.7%, 11.3%, and 11.1% for Southern, Eastern, and Western Africa, respectively) whereas the North America, Western and Southern Europe and Southern Asia contributes the highest (24.3%, 24.4%, 28.9% and 32.3%, respectively) [Bruni et al., 2010].

Figure 1.7: Worldwide prevalence of HPV in cervix of cytologically normal women and Cervical Cancer (CaCx) incidence. The prevalence of HPV in different continents is shown in purple circles and contribution of different High Risk HPV types are shown in the adjacent black circles indicating their respective prevalence in the regions. The color codes assigned to different countries indicates incidence rate (cases/1,00,000) of CaCx, as denoted in the boxes [adapted from Crow JM, 2012].
Figure 1.7
Thus, an interestingly inverse correlation is seen between global prevalence of HPV in cervix and the contribution of HPV16, with the lowest HPV16 proportions in the regions with the highest prevalence (correlation coefficient, -64.8%; P= 0.017) [Bruni et al., 2010]. Undoubtedly the HPV16 is the most prevalent HR HPV, followed by HPV18, throughout the world.

Figure 1.8: Type specific HPV prevalence worldwide (A) and in Asian continent (B). The horizontal bars indicate the prevalence of respective HPV types. The most virulent HR HPV types belonging to Alpha-Genus of papillomaviruses are denoted in Red and Blue, to assign them into respective species 9 and 7, respectively. [adapted from Bruni et al., 2010].

1.6.4 Asian scenario of cervical HR HPV prevalence with special reference to India

The population of Asian continent is ethnically more diverse than the western continents like Europe, North and South America. The prevalence of HPV in normal women population has also varied from time to time in different studies from different Asian countries [Crow JM, 2012; Bruni et al., 2010; Bao YP et al., 2008]. A most recent pooled analysis has reported the overall HPV prevalence in Asian continent is 8.3% (Figure 1.7) which is much lower than the 10.4% prevalence found in the meta-analysis by Bruni et al., 2010 [Crow JM, 2012; Bruni et al., 2010]. In an earlier meta-analysis, the overall prevalence was reported to be 14.4% [Bao YP et al., 2008].
improvement in socio-economic structures beside the differences in cultural practices, ethnicity could be responsible for such differential trend in HPV prevalence. However, among the HPV types, HPV16 was consistently the most common type reported in these studies, with a prevalence of 2.5-2.6% [Crow JM, 2012; Bruni et al., 2010; Bao YP et al., 2008]. The type specific prevalence for most frequent HR HPV types in Asian continent has been shown in Figure 1.8 B.
Among the Asian countries, India is estimated to harbor a significant burden of cervical HPV infection (7.9%) [Bruni L et al., 2014]. However, the age standardized prevalence varied from 7-14% in different studies from India [Bhatla N et al. 2008; Sankaranarayanana R et al., 2005; Sowjanya AP et al., 2005; Laikangban P et al., 2007; Franceschi S et al., 2005]. Moreover, different types of HPV were prevalent in different regions, like HPV16 in Southern, Northern and Eastern India while HPV 18 in North-Eastern states and HPV 52 in South-Eastern states [Bhatla N et al. 2008; Sowjanya AP et al., 2005; Laikangban P et al., 2007; Franceschi S et al., 2005]. The scantiness of population based cervical HPV prevalence studies from different regions of India and the prevalence of HPV and HPV types found in these studies has been demonstrated in Figure 1.9.

The variation of HPV and HPV type prevalence among the different populations might be due to the diverse nature of Indian ethnicity, geography and socio-cultural practices or might be due to the differences in sample size and the methodologies used in the analysis. Despite such discrepancies in HPV prevalence and heterogeneity of HPV type distributions population base large scale studies from India are only a few.

1.7 Association of HLA and IL-1 polymorphisms with persistent HR HPV infection in cervix

More than 80% of cervical HPV infections are cleared of naturally by host immune mechanism, however, about 20% infections persists in the cervix (Figure 1.10A). Such latent infections may eventually transform the normal epithelium to different grades of cervical abnormalities. Persistence of such infections is largely dependent on environmental co-factors, ability of the virus to evade host immune defense and most

Figure 1.10: Diagrammatic representation of step wise development of Cervical Cancer (CaCx) upon infection of HR HPV. A) Role of HPV during development of CaCx. B) Life cycle of HR HPV in conjunction with transformation of normal cervical epithelium. C) Cytological stages of cervix associated with development of Squamous Cell Carcinoma (SCC) [Courtesy Doorbar J, 2005; Snijders et al. 2006].

21
Figure 1.10

A. HR HPV persistence

1. HPV infection
2. Transient infection
3. Normal cervix
4. Inflammation and Atypia (~20%)
5. CIN I
6. CIN II/III
7. Carcinoma

Additive Genetic/Epigenetic alterations 10-12 years

B.

1. HPV infected Cervical Mucosa
2. P97
3. P670
4. CIN I
5. CIN II
6. CIN III
7. CaCx

C. Normal Cervical
   Inflammation
   ASCUS
   LSIL
   HSIL
   Cervical Cancer

Figure 1.10
importantly, host immune-genetic factors against HPV infection/cellular transformation.

Upon infection, the virus specific antigens are processed and displayed to the immune cells by Human Leukocyte Antigen (HLA) Class I/II molecules, for immune surveillance, viral clearance and killing of the infected host or tumor cells [Gough SC et al., 2007]. Among them, HLA I activates the Cytotoxic T-Lymphocytes (CTLs) and Natural Killer cells (NK cells) upon proteasomal degradation and processing of the endogenous peptides within ER (Figure 1.11) [Meleif CJM, 1992; Greenberg PD, 1991]. Whereas, the HLA II follows the lysosomal pathway to display the exogenous antigens on the surface of Antigen presenting cells (APC) for destruction by the CD4+ T-helper cells (Figure 1.11) [Gough SC et al., 2007]. Perturbation of the above mentioned immune-surveillance is rendered by the viral proteins e.g. HR HPV E5, which downregulates the surface expression of HLA I and II proteins, or E7, which represses the promoter of HLA I heavy chain genes, thereby helps the virus to escape immune-attack [Georgopoulos NT et al., 2000; Ashrafi GH et al., 2005; Zhang B et al., 2003]. On the other hand, immune-evasion of the HR HPV infected cells is also facilitated by inefficient presentation of the viral/tumor antigens by HLA molecules. The presence of polymorphism in the antigen binding domain of the HLA I and II molecules result in impaired viral antigen display to the immune cells [Moss DJ et al., 1999; Mueller LP et al., 2002; Thursz MR et al., 1997]. The chromosomal 6p21.31 region containing the HLA class I and II gene loci is extremely polymorphic and the polymorphism varies with the ethnicity of the population, so vary the association of different polymorphic alleles with CaCx susceptibility [Kaufman J et al., 1999]. The HLA Class I polymorphic allele HLA-B*07 has been associated with susceptibility to HR HPV type 16/18 infection in cervix [Bhattacharya P et al., 2007]. In addition to the HLA-B*07 allele, HLA-B*1301 and HLA-B*1801 alleles are also associated with development of CaCx in Indian population [Bhattacharya P et al., 2007; Bhattacharya P et al., 2006]. In African population, predisposition for development of pre-neoplastic cervical lesions and CaCx has been associated with HLA B35 allele [Muchiri L et al., 2012].
Figure 1.11 A) Diagrammatic representation of the HLA region on chromosome 6p21. B) More detailed diagrams of common alleles within the HLA class I and class II region C) Representation of endogenous antigen presentation by HLA class I molecules (including HLA-A,-B and –C). Endogenous antigen generated in the cytosol is degraded within the proteasomes and then transported into the rough endoplasmic reticulum (RER) through the TAP1/TAP2 complex. Antigen is then bound by HLA class I molecules in connection with β2M and then exported to the cell surface for recognition by CD8+ T cells and natural killer (NK) cells. Exogenous antigen presentation by HLA class II classical molecules (including HLA-DR and –DQ) is also represented. Exogenous antigen is imported into the cell and then enters the endocytic pathway (encompassing the early endosome, late endosome and lysosome) where the antigen is degraded. At the same time HLA class II molecules complexed with the invariant chain move from the RER where they are synthesized to the endocytic pathway. As the HLA class II/Invariant chain complex moves into the increasing more acidic compartment of the endocytic pathway the invariant chain is digested, leaving only CLIP bound. The CLIP is then replaced with degraded antigen and then the HLA class II molecule/antigen complex is exported to the surface of the cell for presentation to CD4+ T helper (Th) cells.

PSMB = Proteasome subunit B –type, TAP = Transporters associated with antigen presentation, β2M = β2 Microglobulin, CLIP = class II associated invariant chain. [Adapted from: Gough SC et al., 2007].
Figure: 1.11

Figure: 1.11
Similarly, the presence of HLA A*0201 or B*4402 or Cw*0501 alleles, either singly or in haplotype combination with other HLA II alleles was associated with increased risk of CaCx development in American population [Madeleine MM et al., 2008]. Interestingly, a study from China showed the association of HLA-B*07 allele with maximum risk of familial CaCx development, in presence of HR HPV [Qiu X et al., 2011]. In contrary, the HLA Bw7, Cw6 and Cw0701 alleles showed protection against CaCx development [Muchiri L et al., 2012; Madeleine MM et al., 2008].

1.7.2 Association of HLA Class II polymorphism with CaCx susceptibility

For clearance of HR HPV infection, HLA II mediated immune-activation of T-helper cells seems to be utmost important, failure of which may predispose the individual to persistent infection, followed by neoplastic transformation. The association between HLA Class II polymorphisms and CaCx has been widely studied in different populations. In African population, the HLA-DR1 and DQ5 alleles showed increased susceptibility for pre-neoplastic lesions of cervix, whereas, the HLA-DR1 allele was also associated with CaCx development [Muchiri L et al., 2012]. Similarly, the individuals with DQB1*0301 allele, either singly or in haplotype combination with DRB1*0401 showed elevated risk for CaCx development [Cuzick J et al., 2000]. Moreover, presence of DRB1*1501 allele, either singly or in haplogroup with DQB1*0602 allele rendered increased susceptibility to HPV16 mediated CaCx development [Cuzick J et al., 2000]. Interestingly, the risk association between HLA DRB1*15 allele with HPV16 variants has been found during CaCx development [de Araujo Souza PS et al., 2008]. The risk association of HLA-DQB1*03, HR HPV infection and CaCx development have been extensively reported from different countries, including India [Wank R et al., 1991; Nawa A et al., 1995; Montoya L et al., 1998; Gregoire L et al., Int J Cancer, 1994; Allen M et al., 1996; Apple RJ et al., 1994; Bhattacharya P et al., 2006]. The antigen HLA-DQw3, encoded by this polymorphic allele inefficiently displays the viral antigens to antigen presenting cells (APC), thereby predisposes the individual to CaCx development [Wank R et al., 1991].
However, these studies did not address the correlation between polymorphism of HLA Class II allele(s) and clearance of HR HPV infection from cervix.

1.7.3 *Association of IL-1 promoter polymorphism with CaCx susceptibility*

Following HR HPV infection, chronic inflammation is the initial histo-pathological stage of transforming cervical epithelium. Among the pro-inflammatory cytokines produced by the host monocytes and tissue-macrophages to modulate the inflammatory signals against viral infections, IL-6 and IL-1β are most important [Bird S et al, 2002; Diehl S et al., 2002]. Increased expression of IL-6 in cervico-vaginal secretion of CaCx patients is associated with severity of the disease as higher IL-6 level promotes tumor angiogenesis and thereby promotes tumorigenesis [Wei LH et al., 2003]. Presence of A>G polymorphism in the transcription factor binding site located in 2nd intron of IL-6 gene is associated with higher expression of IL-6 and with increased risk for CaCx development [Nogueira de Souza NC et al., 2006]. Interestingly, persistence of HPV DNA in women with history of abnormal cervical cytology has been associated with the level of IL-6 in plasma [Rosa MI et al., Arch Gynecol Obstet, 2012].

Similarly, the increased secretion of IL-1β has been reported to be associated with CaCx [Hall SK et al., 2004]. The -511 C/T and -31 T>C polymorphisms located in the promoter region of IL-1β, adjacent to the TATA-box, is associated with increased intracellular production of IL-1β and increased risk of CaCx development [Al-Tahhan MA et al., 2011; Sobti RC et al, 2008; Qian N et al., 2010]. The association of IL-1β -511 C/T polymorphism with risk of CaCx development has been well documented in various studies from India, Korea and Egypt  [Kang S et al., 2007; Singh H et al., 2008; Al-Tahhan MA et al., 2011]. Most importantly, increased transcription was reported when IL-1β promoter contained the -511T allele in haplogroup with -31C allele [Chen H et al., 2006]. However, to the best of our knowledge, the risk association of IL-1β promoter polymorphism with clearance of HR-HPVs and remission of HPV associated infection in cervix has not been reported earlier.
### Table 1.6: Epidemiology of Cervical Cancer: Global perspective (adapted from Maura L. Gillison, Int. J Cancer, 2014).

<table>
<thead>
<tr>
<th>Epidemiologic trait</th>
<th>Cervical Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etiology</td>
<td>Oncogenic HPV infection only with or without intervening cofactors</td>
</tr>
<tr>
<td>Number of cases worldwide (2008)</td>
<td>530,000</td>
</tr>
<tr>
<td>Quality and amount of accumulated evidence for HPV role</td>
<td>Large, robust, diverse in study designs and consistent across geography and study populations</td>
</tr>
<tr>
<td>Etiological HPV fraction</td>
<td>100%</td>
</tr>
<tr>
<td>Number of cases attributed to HPV worldwide -2008</td>
<td>530,000</td>
</tr>
<tr>
<td>Developing/developed burden of HPV related cases</td>
<td>453,000/77,000</td>
</tr>
<tr>
<td>Burden of HPV-related cancer cases relative to all cancers attributable to infectious agents</td>
<td>48.2% (530,000/1,100,000 female cases)</td>
</tr>
<tr>
<td>Trends</td>
<td>Decreasing in most but not all developed and developing countries</td>
</tr>
<tr>
<td>HPV 16 relative contribution</td>
<td>61%</td>
</tr>
<tr>
<td>HPV 18 relative contribution</td>
<td>10%</td>
</tr>
<tr>
<td>Relative contribution of HPV 16 and 18</td>
<td>71%</td>
</tr>
<tr>
<td>Relative contribution of other types</td>
<td>Between 2 and 6% each (31, 33, 35, 39, 45, 52, &amp; 58 among many others)</td>
</tr>
<tr>
<td>Evidence for type-specific carcinogenicity</td>
<td>For all High Risk types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59</td>
</tr>
</tbody>
</table>

#### 1.8  **HR HPV and Cervical Cancer Development**

**1.8.1 Cervical Cancer: Incidence and HR HPV prevalence**

Cancer of the uterine cervix (CaCx) is the third most common cancer among women, with an estimated 5,30,00 cases worldwide in 2008 and 83,195 new cases and 35,673
deaths only in 2012 (Table 1.6) [Gillison ML et al., 2014]. The majority of CaCx cases are Squamous Cell Carcinoma (SCC) followed by Adenocarcinomas. Worldwide, mortality rates of CaCx are substantially lower than incidence, with mortality : incidence ratio of 50.3% [Ferlay J et al., GLOBOCAN 2012]. In Figure 1.7 the global incidence rate of CaCx has been shown which varies among continents. In 2010, 76% of global CaCx cases (3,44,535 of 4,53,970) occur in developing countries and 17% (76,100) of these cases were in sub-Saharan Africa. The number of CaCx cases has been increasing all over the world except in developed countries. The incidence rate has been constant in the East and South Asia, Eastern Europe, and Southern Latin America. Infection of HPV, more precisely HR HPVs are associated as necessary etiological factor with CaCx development throughout the world, as nearly all CaCx cases were found to be positive for HPV DNA (Table 1.6) [Gillison ML et al., 2014]. Among the HR types HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 are most commonly associated with CaCx but majority of them are contributed by HPV16 and 18 (combined relative contribution 71%; HPV16 relative contribution 61%; HPV18 relative contribution 10%) [Gillison ML et al., 2014].

In India, CaCx is the 2nd most frequent cancer among women between 15 and 44 years of age, killing 67,477 women every year [Bruni L et al., 2014]. Estimated 1,22,844 women are diagnosed with CaCx every year. HPV16 and HPV18 constitute more than 84% of the HR-HPV types present in the CaCx in India and HPV16 alone contribute 69% of it [Bruni L et al., 2014; Mazumder D et al., 2011]. In the pre-neoplastic lesions of cervix (Cervical Intraepithelial Neoplasia, CIN) the prevalence of HPV16 and 18 are relatively higher than the other HR HPV types. In low-grade CIN (CIN-I) the combined prevalence of HPV16 and 18 is about 28%, whereas, in high-grade lesions (CIN-II-III) the prevalence is nearly 56% [Bruni L et al., 2014]. Therefore, it is legitimate to concentrate the research interest on HR HPVs, especially on HPV16, for its etiological role and virulence during CaCx development.
1.8.2 Role of HR HPV in CaCx development

HR HPVs, being a necessary etiological factor, develops CaCx as a result of infection in normal cervical epithelium, its persistence and subsequent violation of normal tissue physiology by deregulation of cell cycle. The cervix is covered by two types of epithelium, the outer portion i.e. the ectocervix is covered by the stratified, non-keratinising squamous epithelium, whereas, the inner endocervix is covered by tall, glandular columnar epithelium (Figure 1.12) (Sankaranarayanan et al., 2003). This squamo-columnar junction, containing most of the stem cells, is called the transformation zone because of its susceptibility to neoplastic events (Moscicki et al., 2006). The infection of HR HPV targets this squamo-columnar junction to propagate (Figure 1.12).

The life cycle of HR HPVs in context of CaCx development has been demonstrated in Figure 1.10B.

Figure 1.12: Schematic diagram of cervical epithelium showing the squamous, columnar cell layers and the squamo-columnar transformation zone (TZ) (A), infection of HR HPV in the basal cells of TZ (B) and transformation of epithelium (C).
1.8.2A Transient infection of HR HPV in cervical epithelium

- **Infection of HR HPV in normal cervical epithelium**
  The basal layer of stratified squamous epithelium of uterine cervix is infected by HPV through micro-wound. Most studies have suggested that the virus infects an epithelial stem cell via heparin sulphate or by endocytosis of clathrin coated vesicles [Egawa 2003; Schmitt et al. 1996; Giroglou et al. 2001; Joyce et al. 1999; Culp and Christensen 2004; Day et al. 2003; Selinka et al. 2002].

  Following entry of HPV into the basal layer of the epithelium, uncoating of the virus is facilitated by the disruption of intracapsomeric disulphide bonds in the reducing environment of the cell, allowing the viral DNA to be transported into the nucleus [Li et al. 1998].

- **Maintenance of viral genome**
  Following infection and uncoating inside the basal cells of the epithelium, the virus maintains its genome as a low copy number episome by synergizing its replication with cell division [Wilson et al. 2002; You et al. 2004]. The viral genome is maintained in the basal layer at around 10–200 copies per cell by means of poorly characterized viral early gene expression (Figure 1.10B). It is thought that the E1, E2, E6 and E7 proteins are expressed at low level for maintenance of the viral DNA as an episome [De Geest et al. 1993; Stanley et al. 1989]. However, the role of E6 and E7 to basal cell proliferation is uncertain and E1, E2 alone is thought to be sufficient for maintenance of the episomal genome [Zhang et al. 1999].

- **Proliferative phase**
  Normally the basal cells exit from cell cycle soon after migrating into the spinous layer and undergoes terminal differentiation. But upon HPV infection, cell cycle progression is promoted by the viral E7 and E6 gene expression and terminal differentiation is retarded in these cells (Figure 1.10B) [Sherman et al. 1997]. The E6 and E7 are transcribed from the early promoter of HPV genome (p97) as a bi-cistronic...
mRNA, can associate with regulators of the cell cycle and stimulate cell cycle progression (Figure 1.5C) [Stacey et al. 2000; Munger et al. 2001].

- **Genome amplification**
  Amplification of HPV genome begins in a subset of cells in the proliferative compartment and requires expression of all early gene products involved in viral DNA replication (i.e., E1, E2, E4 and E5) by up-regulation of the late promoter (p670) within the E7 open reading frame (Figure 1.5C & Table 1.4) [Fehrmann et al. 2003; Genther et al. 2003; Middleton et al. 2003; Peh et al. 2004]. The binding of E2 to the HPV upstream regulatory region is necessary for viral DNA replication, and recruits the E1 DNA helicase to the viral origin of replication (Table 1.4). Throughout the virus life cycle, the relative levels of different viral proteins are controlled by promoter usage and by differential splice site selection, with an increase in the level of E1 and E2, allowing an increase in viral copy number in the upper epithelial layers [Ozbun and Meyers 1998a; Schwartz 2008]. The newly replicated genome serves as templates for further expression of E1 and E2, which would facilitate additional amplification of viral genomes and in turn, further expression of the E1 and E2 replication proteins [Middleton et al. 2003].

- **Virus synthesis**
  The two structural proteins of HPV, viz. L1 and L2 are expressed in the upper layers of infected tissue once genome amplification phase has been completed (Figure 1.10B) [Ozbun and Meyers 1998b]. The L1 and L2 are transcribed from the late promoter (p670) and L1 is expressed after L2. The assembly of infectious virions occurs in a subset of upper layer of the epithelial cells that express E4 (Figure 1.10B) [Doorbar et al. 1997; Florin et al. 2002]. Being a non-lytic virus, papillomaviral virions are not released until the infected cells reach the cornified surface of the cervical epithelium for exfoliation. During exfoliation the virus eventually escape from the infected cells, survive extra-cellularly and reinfection.
According to Bethesda, 2001 classification, abnormal cellular types associated with transient HR HPV infection in cervix are (Figure 1.10C):

1. **Inflammation:**
   
i. Cells with minimal nuclear enlargement (<2X intermediate).
   
ii. Smooth nuclear membranes.
   
iii. Fine, uniform chromatin.
   
iv. Nucleoli single or multiple, can be prominent.
   
v. Cytoplasm may show Polychromasia, vacuolization and perinuclear halos.

2. **Atypia:**
   
i. Nuclei are 2-1/2 to 3 times larger than the size of an intermediate cell nucleus.
   
ii. Slightly increased N:C ratio.
   
iii. Variation in size/shape and binucleation may occur.
   
iv. Smooth nuclear membrane outlines.
   
v. Normochromic with even distribution of chromatin.
   
vi. Cytoplasm usually mature.
   
vii. Cytoplasm can also show metaplastic changes.

Commonly two types of atypia found, on the basis of the involvement of cell types-

a. **Atypical Squamous Cell of Undetermined Significance (ASCUS):**
   Atypical ectocervical Cells.

b. **Atypical Granular Cell of Undetermined Significance (AGUS):**
   Atypical endocervical Cells.

Though inflammation or atypia are cytologically distinct from normal cytology, they remain clinically symptomless, hence commonly grouped together as **ASYMPTOMATIC INFECTION.**

**1.8.2B** Persistent infection of HR HPV in cervical epithelium
The transformation of infected cervical epithelium is instigated by deviation from normal life cycle of HR HPV. The initial step of transformation is complex and includes abrogation of the normal cell cycle control by disruption of key regulatory genes viz p53, pRb, p107, p130, p21, p27 etc. by the viral oncoproteins E6, E7 and E5, as mentioned in Table 1.4. [Munger et al. 1989; Jones et al. 1997a; Zerfass-Thome et al. 1996; Huibregtse et al. 1991; Scheffner et al. 1990; Scheffner et al. 1993]. The deviation from normal life cycle of HR HPV is associated with integration of the viral genome into host genome through disruption of E2 gene and production of E7 and E6 in excess due to upregulation of the early (p97) promoter (Figure 1.10 A & B). The control of gene expression is also affected by the methylation profile of the early-late promoters and the regulatory enhancer sequence.

The deregulation of cell cycle by the HR HPV E7, E6 and E5 oncoproteins have been described below.

**Role of E7 in CaCx development**

![Figure 1.13: Disruption of Cellular pathways by E7 oncoproteins of HR HPVs. The cellular targets of E7 have been shown in blue and as a result the altered expression of the key cell cycle regulators has been shown within green boxes. The consequences cellular pathways have been indicated in brown boxes (adapted from Moody and Laimins, 2010).](image)
The E7 from HR HPV triggers its oncogenicity by protein-protein interactions to several cellular factors, disobeying the normal proliferation control of the cell and inducing hyper-proliferation (Figure 1.13). It chiefly targets the key tumor suppressor proteins pRb and its related family members p107, p130 through the conserved amino-terminal LXCXE motif [Munger et al. 1989]. The binding of E7 to Rb disrupts Rb–E2F complexes, resulting in the constitutive expression of E2F-responsive genes, such as Cyclin A and Cyclin E, promoting G1-S phase transition, premature S-phase entry and DNA synthesis [Chellappan et al. 1992; Cheng et al. 1995; Zerfass et al. 1995]. Additionally, the carboxy-terminus of HR E7 proteins bind to p21 and p27, neutralizing their inhibitory effects on Cyclin A and Cyclin E-associated kinase activities [Jones et al. 1997a; Zerfass-Thome et al. 1996]. Thus, CDK2 activity remains high in E7-expressing cells despite high levels of p21 [Funk et al. 1997; Jones et al. 1997a]. HR E7 can induce tyrosine dephosphorylation of CDK2 by increasing the levels of the CDC25A phosphatase, promoting its activation [Blomberg and Hoffmann 1999; Katich et al. 2001; Nguyen et al. 2002]. E7 also induces proteasomal degradation of pRb family members’ p107, p130 through the ubiquitin-dependent pathway [Boyer et al. 1996; Jones et al. 1997b]. The interaction between E7–Rb–HDAC is essential for episomal maintenance and for maintaining an S-phase environment on differentiation [Longworth and Laimins 2004; Longworth et al. 2005]. HR E7 proteins achieve this by binding to HDACs and facilitate HDAC removal at other promoters to activate transcription [Longworth and Laimins 2004; Longworth et al. 2005]. E7 can also promote telomere maintenance in the absence of E6 [Stoppler et al. 1997]. Similar to E6, E7 also induces genomic instability through activation of the ATM–ATR pathway [Moody and Laimins 2009]. Degradation of Claspin, a key regulator of the ATR–CHEK1-DNA damage-signaling pathway, that is activated in response to replication stress is induced by E7 and stimulating aberrant mitotic entry in the presence of DNA damage [Spardy et al. 2009].

**Role of E6 in CaCx development**

The consequence of deregulated cell-cycle control by E7-pRb interaction is an increase in p53 level to induce cell cycle arrest and apoptosis [Demers et al. 1994;
Eichten et al. 2004; Jones et al. 1997a]. The HR HPVs subvert these signals by the E6 oncoproteins which interacts with different cellular proteins to affect various signaling pathways. To abrogate the growth arrest E6 forms a trimeric complex with E6AP (E6-associated proteins) and p53 which leads to ubiquitinylation and degradation of p53 (Figure 1.14) [Huibregtse et al. 1991; Scheffner et al. 1990; Scheffner et al. 1993]. E6 also interact with p53 to interfere with its transcription inducer role by blocking the DNA-binding activity [Lechner and Laimins 1994]. By interacting with two acetyltransferases p300 and CREB binding proteins (CBP), E6 blocks their ability to acetylate and degrade p53, thereby increase its stability [Patel et al. 1999; Zimmermann et al. 1999]. To prevent apoptosis of the infected cells E6 targets Bax, Bak and caspase-8 proteins of the apoptotic pathway in addition to the induction to

Figure 1.14: Disruption of Cellular pathways by E6 oncoproteins of HR HPVs. The cellular proteins that are down regulated by E6 have been shown in blue and the upregulated proteins have been shown within green boxes. The consequences of altered cellular pathways have been indicated in brown boxes (adapted from Moody and Laimins, 2010).
p53 degradation, prevention of p53 acetylation and blocking its transcriptional activity [Howie et al. 2009; Moody and Laimins 2010]. By the interaction of HR HPV E6 proteins with PDZ-domain containing proteins like MAGI-1, MUUP1 favors loss of viral episomes and frequent integration of the viral genome into host chromosome, promoting genomic instability [Thomas et al. 2008; Lee et al. 2007]. In the apoptotic resistant cells, E6 further induces genomic instability by inducing numerous mitotic defects, such as multipolar mitoses, anaphase bridges, aneuploidy and also by activating the ATM-ATR pathway [Duensing et al. 2000; Duensing et al. 2009; White et al. 1994; Moody and Laimins 2009]. In an extra step towards immortalization the E6 activates telomerase reverse transcriptase (TERT) and telomerase in the infected cells [Howie et al. 2009; Wise-Draper et al. 2008]. Finally, the interaction of E6 with the focal adhesion kinase (FAK) protein paxillin and the extracellular matrix protein fibulin prevents anoikis and favoring anchorage-independent growth of the HR HPV transformed cells [Moody and Laimins 2009].

❖ Role of E5 in CaCx development

![Diagram](image)

Figure 1.15: The E5 oncoproteins of HR HPVs augments the transforming activity of E6 and E7 (adapted from Moody and Laimins, 2010).
Although the primary transforming activity of HR HPVs is rendered by E6 and E7, E5 enhances the transforming activity of E6 and E7 (Figure 1.15) [Moody and Laimins 2009]. Moreover, it interacts with cell membrane receptors such as EGFR (epidermal growth factor receptor) and PDGFR (platelet derived growth factor receptor) and G protein-coupled endothelin receptor (ETA)/ET1 which stimulates proliferation of infected cells, promotes fusion between cells and also induces evasion of immune response [Fernandes JV et al., 2013; Venuti A et al., 2011].

Cytological abnormalities associated with development of CaCx due to persistent HR HPV infection in cervix are (Figure 1.10C):

1. **Low-grade Squamous Intraepithelial Lesions (LSIL):** This is the early pre-neoplastic lesion of cervix and is synonymous to the histo-pathological lesion CIN I (Cervical Intraepithelial Neoplasia-I). Characteristic features of LSIL are:
   
   - **i.** Cells are single or in sheets.
   - **ii.** Usually mature cells (Superficial intermediate) with polygonal cell borders.
   - **iii.** May have perinuclear clearing (Koilocytotic halo).
   - **iv.** Nuclei are at 3X area of an intermediate nucleus with increased N: C ratio.
   - **v.** Moderate variation in size/shape.
   - **vi.** Binucleation or multinucleation frequent.
   - **vii.** Slightly irregular nuclear membrane.
   - **viii.** Hyperchromasia with uniform chromatin, chromatin smudging.

2. **High-grade Squamous Intraepithelial Lesions (HSIL):** This pre-neoplastic lesion of cervix is synonymous to the histo-pathological stage of CIN II-III and can be characterized by:
   
   - **i.** Cells are seen as single cells or sheets or in syncytial like groups.
ii. Usually immature cells.
iii. Overall size cell size smaller than LSIL.
iv. Cytoplasm is lacy and delicate.
v. Dense, metaplastic cytoplasm.

3. **Squamous Cell Carcinoma (SCC):** The invasive carcinoma of cervical squamous Cell origin.
   
i. Single cell or in syncytial-like aggregates.
   
   ii. Cellular features of HSIL with Prominent acronucleoli, markedly irregular chromatin.
   
   iii. Tumor diathesis often present.

1.9 **Genetic and epigenetic profile of HR HPV during CaCx development**

Although persistent infection of HR HPVs, predominantly the infection of HPV16 is necessary for development of CaCx, additional cytogenetic and molecular alterations takes place, as indicated in section 1.8.2B and Figure 1.10A. In addition to these alterations at host cell level, the HPV genome characteristically changes throughout the developmental stages of CaCx, starting from its initial infection to the latent infection to the pre-neoplastic lesions to the invasive tumor. Even such changes in HPV genome has also been seen with progression of the tumor, i.e. in cervical lesion of CaCx patient, before and after therapy. Depending on mechanisms involved, they can be broadly categorized into:

- Change in genetic profile of HR HPV
- Change in epigenetic profile of HR HPV

1.9.1 **Change in genetic profile of HR HPV:**

The change in genetic profile of HR HPV includes the variation in sequence of different early and late genes or in the LCR sequence; variation in the viral
Table 1.7: Sequence variations in HPV16 genome, as observed in CaCx samples, and its biological impact (Ref. Mazumder D et al., 2011; Pillai MR et al., 2009).

<table>
<thead>
<tr>
<th>HPV 16 Genomic Variant</th>
<th>% prevalence in CaCx</th>
<th>Biological functions affected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In E6</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A131G</td>
<td>2.5%</td>
<td>p53 binding &amp; degradation, B &amp; T cell epitope</td>
</tr>
<tr>
<td>G145T</td>
<td>13.1%</td>
<td>p53 binding &amp; degradation, B &amp; T cell epitope</td>
</tr>
<tr>
<td>T178G</td>
<td>1.7%</td>
<td>T cell epitope</td>
</tr>
<tr>
<td>C335T</td>
<td>12.1%</td>
<td>p53 binding &amp; degradation, T cell epitope, E6 transcriptional transactivation</td>
</tr>
<tr>
<td>T350G</td>
<td>72.3%</td>
<td>E6 transcriptional transactivation,,T cell epitope, p53 degradation</td>
</tr>
<tr>
<td>T521G</td>
<td>1.45%</td>
<td>E6 transcriptional transactivation, p53 binding and degradation, B &amp; T cell epitope</td>
</tr>
<tr>
<td><strong>In E7</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A647G</td>
<td>1.7%</td>
<td>DNA synthesis, E2F-pRb dissociation, pRb binding, NLS</td>
</tr>
<tr>
<td>T732C</td>
<td>9%</td>
<td>Metal binding domain, pRb binding, E2F-pRb dissociation</td>
</tr>
<tr>
<td>G823A</td>
<td>1.7%</td>
<td>Metal binding domain, E2F-pRb dissociation</td>
</tr>
<tr>
<td>A826T</td>
<td>1.2%</td>
<td>Metal binding domain, E2F-pRb dissociation</td>
</tr>
<tr>
<td>T846C</td>
<td>1.7%</td>
<td>Metal binding domain, E2F-pRb dissociation</td>
</tr>
<tr>
<td><strong>In L1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5600A</td>
<td>0.86%</td>
<td>Nonsence</td>
</tr>
<tr>
<td>G5829A</td>
<td>1.6%</td>
<td>B cell epitope</td>
</tr>
<tr>
<td>C5862T</td>
<td>12.2%</td>
<td>T &amp; B cell epitope</td>
</tr>
<tr>
<td>G5871T</td>
<td>0.24%</td>
<td>B cell epitope</td>
</tr>
<tr>
<td>A6021G</td>
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<td>L1-L1-interface</td>
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<tr>
<td>G6024A</td>
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<td>Assembly</td>
</tr>
<tr>
<td>C6163A</td>
<td>13.9%</td>
<td>B cell epitope &amp; assembly</td>
</tr>
<tr>
<td>Position</td>
<td>Frequency</td>
<td>Function</td>
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<tr>
<td>A6166T</td>
<td>0.31%</td>
<td>B cell epitope</td>
</tr>
<tr>
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<td>2.2%</td>
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<tr>
<td>A6178C</td>
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<tr>
<td>C6240G</td>
<td>94%</td>
<td>Assembly</td>
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<tr>
<td>C6388T</td>
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<td>B cell epitope</td>
</tr>
<tr>
<td>A6432G</td>
<td>85%</td>
<td>L1-L1 interface, B cell epitope</td>
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<td>C6502T</td>
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<td>Assembly, B cell epitope</td>
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<td>T6560A</td>
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<td>B cell epitope</td>
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<td>Nonsense, B cell epitope</td>
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<tr>
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<td>100%</td>
<td>B Cell epitope</td>
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<tr>
<td>6950delGT</td>
<td>100%</td>
<td>B Cell epitope</td>
</tr>
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<td>NLS</td>
</tr>
<tr>
<td>A7132C</td>
<td>0.4%</td>
<td>NLS</td>
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**In LCR**

<table>
<thead>
<tr>
<th>Position</th>
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<th>Function</th>
</tr>
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<td>GRE/1, YY1</td>
</tr>
<tr>
<td>C7395T</td>
<td>6.8%</td>
<td>GRE/1</td>
</tr>
<tr>
<td>A7485C</td>
<td>18.6%</td>
<td>GRE/2, YY1</td>
</tr>
<tr>
<td>G7489A</td>
<td>18.6%</td>
<td>GRE/2, YY1</td>
</tr>
<tr>
<td>C7689A</td>
<td>18.6%</td>
<td>TEF-1</td>
</tr>
<tr>
<td>T7714G</td>
<td>13.6%</td>
<td>NF1</td>
</tr>
<tr>
<td>A7729C</td>
<td>18.6%</td>
<td>NF1, YY1</td>
</tr>
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<td>6.8%</td>
<td>TEF-1</td>
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<td>13.6%</td>
<td>YY1</td>
</tr>
<tr>
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<td>13.6%</td>
<td>NF1, YY1</td>
</tr>
<tr>
<td>G7826A</td>
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<td>Oct</td>
</tr>
<tr>
<td>C7886G</td>
<td>6.8%</td>
<td>YY1</td>
</tr>
</tbody>
</table>
copy number, commonly known as viral load; and change in physical status of HPV genome i.e. Episomal/Integrated/Mixed. All these genetic changes are stable and impact on virulence of the virus and thereby on pathogenesis of the disease.

1.9.1A Variation in sequence of HR HPV genome

Among the HR HPV types prevalent throughout the World, most extensive genomic variability is seen in HPV16, followed by HPV18 and HPV45. Based on the sequence variation of the L1, L2 and LCR regions of HPV16, six naturally occurring lineages have been identified. These lineages are European (E), Asian (As), Asian-American (AA), North American (NA-1), African-1 (Af-1) and African-2 (Af-2) [Giannoudis et al. 2001; Yamada et al. 1997]. The prototype sequence of HPV16 belongs to the European lineage and is used as reference sequence for comparison with other lineages of HPV16. Although the E2, E4, E5, E6 and E7 genes of HPV16 further shows intratypic sequence variation, the E6 gene sequence show most extensive variations among them [Yamada et al. 1997]. Thus, variants within the lineages are grouped on the basis of nucleotide substitutions of E6 genes. For example, the European prototype (Ep) variant having complete sequence homology to the sequence of HPV16 prototype E6. Another common example of European variant is E-G350 which belongs to the European variant with a T to G transversion at nucleotide position 350. The oncogenicity of different HPV variants differs with ethnicity and geography. The HPV16 E6 and E7 variants were associated with the increased risk of CIN and CaCx [Burd EM. 2003; Giannoudis et al. 2001; Matsumoto et al. 2000; Zehbe et al. 1998a]. In India, the HPV16 Ep-G350 variant was most common in CaCx, followed by the European prototype (Ep) and AA variants [Mazumder DI et al., 2011; Pande S et al., 2008].

In CIN and CaCx samples, non-synonymous sequence changes were present in E6, E7, L1 and LCR regions of HPV16 [Mazumder DI et al., 2011; Pillai MR
et al., 2009; Pande S et al., 2008]. The variation in E6 sequence has been predicted to affect its interaction with B and T-cell epitopes, binding with E6AP, binding and degradation of p53 (Table 1.7) [Pillai MR et al., 2009]. The E7 variant has been predicted to affect the pRb-binding, E2F-pRb dissociation, Nuclear Localization Singaling (NLS) (Table 1.7) [Pillai MR et al., 2009]. On the other hand, the sequence variation of L1 gene might lead to the amino acid change related to the induction of specific immune responses, disruption of capsid pentamer formation and L1 protein interface interaction (Table 1.7) [Pillai MR et al., 2009]. Whereas, the variation in LCR has been predicted to interfere with binding of various transcription factors like YY1, NF1, TEF-1, Oct-1, thereby differentially regulates the transcription of early proteins in CIN and CaCx samples (Table 1.7) [Mazumder DI et al., 2011].

1.9.1B Variation in physical status of HR HPV

![Diagram of HPV16 E2 protein](image)

Figure 1.16: The diagrammatic structure of HPV16 E2 protein, showing its functional domains. The E2 ORF spanned within the viral genome has been indicated in nucleotide numbers underneath.

The HR HPV integrates into the host genome through disruption of the hinge region of its E2 ORF (Figure 1.16) [Dell et al. 2001; Hegde, 2002]. Disruption of E2 deregulates the transcription of E6/E7 oncoproteins, resulting in induction of HR HPV oncoprotein mediated transformation [Jeon et al. 1995]. The HPV integration occurs throughout the host genome but the preferential site of integration is common fragile site (CFS) [Yu et al. 2005]. About 48% of the HPV16 and 68% of the HPV18 integration occurs within CFS [Thorland et
al. 2003]. Thus, the change of HR HPV physical status from episomal to mixed (episomal + integrated) to fully integrated is associated with CaCx development. The pre-neoplastic lesions (CIN) were mostly associated with episomal HPV genome (45-50%) and lower frequency of mixed (about 20%) or integrated (30%) forms [Mazumder DI et al., 2011; Jeon and Lambert 1995; Kulmala et al. 2006; Peitsaro et al. 2002]. Whereas, in CaCx HPV16 was predominantly integrated (>60%) with the episomal and mixed form in considerably lower percentage [Mazumder DI et al., 2011]. Exceptionally, predominance of exclusively episomal form of HPV16 (63-81%) was reported in few studies [Bhattacharjee et al., 2006a; Cullen et al. 1991]. Moreover, the physical status of HR HPV has also been studied in cervical specimens of post-therapy CaCx patients for their possible association with disease prognosis [Badaracco et al. 2002; Lindel et al. 2006; Shin HJ et al., 2014]. However, the physical status of HPV16 in asymptomatic infections is largely unknown.

1.9.1C Variation in HR HPV copy number

The copy number of HR HPVs successively increases after initial infection in cervical epithelium. Initially, the virus maintains low copy number episomal form in asymptomatic infections which may change upon integration of the virus during CaCx development. HPV16 and HPV18 were present in high copy number than other HR HPV types in cervical lesions and the copy number increases with increasing epithelial abnormalities, from normal cytology to CIN to CaCx [Singh A et. al, 2009; Swan DC et al., 1999]. Interestingly, the cervical lesions with integrated HPV16 showed rapid progression to malignancy compare to the lesions with episomal HPV16 [Peitsaro P et al., 2002]. Whereas, the virus in mixed form showed more rapid progression with increase in integrated HPV16 copies [Peitsaro P et al., 2002]. However, the change of HPV copy number from initial asymptomatic infection to the successive stages of CaCx development has not been reported from India. Interestingly, presence of HPV in post-therapy cervix in high copy
number has been associated with poor disease prognosis [Singh RK et al., 2006]. But any prospective study on change of HPV16 copy number in cervical lesions of CaCx patients, before and after therapy is lacking. Such study might be useful for early disease prognosis.

1.9.2 Change in epigenetic profile of HR HPV

The two promoters (p97 and p670) and the upstream enhancer region of the HR HPV genome is under the influence of DNA methylation (CpG methylation) mediated down-regulation of early and late gene transcription, respectively. The CpG methylation in HPV16 and HPV18 genomes occur more often in LCR and part of L1 ORF than in any other parts [Badal et al. 2003; Badal et al. 2004]. Differential methylation of the CpG islands in the early-promoter (p97) and enhancer regions of LCR has been seen in the premalignant and malignant cervical lesions [Badal et al. 2003; Bhattacharjee et al. 2006b; Kalantari et al. 2004]. In the CIN lesions, lower methylation of LCR was found, however, in CaCx samples the methylation frequency varied widely among different investigators [Badal et al. 2003; Bhattacharjee et al. 2006b; Kalantari et al. 2004]. The in vitro analysis showed methylation in the CpG of E2BS prevents E2 protein binding, thereby modulating E2 transcriptional activator function [Thain et al. 1996; Kim et al. 2003]. Interestingly, during transformation of pre-neoplastic lesions differential methylation of the four E2BSs was reported by Vinokurova et al. [Vinokurova et al., 2011]. When methylation of HPV16 genome was analyzed in concurrence of the viral physical status, inversely correlation between viral methylation and integration status was observed [Mazumder D et al., 2011]. With development of CaCx, decreased LCR methylation was observed irrespective of the physical state of the virus [Mazumder D et al., 2011]. However, the frequency of methylation of enhancer and promoter were similar. Badal et al. correlated hypomethylation of LCR with CaCx progression [Badal
et al. 2003]. However, the methylation profile of the late-promoter has rarely been studied.

### 1.10 HR HPV in Circulating Tumor Cells (CTC): Prognostic implications

![Diagram](image)

**Figure 1.17: Circulating tumor cell and metastatic process.** EMT: Epithelial-mesenchymal transition; CTC: Circulating tumor cell; DTC: Disseminated tumor cell; MET: Mesenchymal-epithelial transition [adapted from: Park Y et al., 2011].

#### 1.10.1 Origin and cellular features of Circulating Tumor Cells (CTC)

The presence of Circulating Tumor Cells (CTC) in peripheral blood circulation of cancer patients, especially with metastatic tumor, has been reported in various studies [Wittekind C et al., 2005; Friedl P et al., 2003]. The
proliferative tumor mass reduces oxygen supply in the cells of tumor core [Hanahan D et al., 2000]. The interstitial and inflammatory mechanisms are activated for angiogenesis to reduce this oxygen stress in the tumor mass [Hanahan D et al., 2000]. The tumor cells infiltrate into these new born fragile blood vessels and are carried to distant organs via blood circulation (Figure 1.17). Such intravascular filtration and metastasis were previously considered as late event of tumor progression [Wittekind C et al., 2005; Friedl P et al., 2003]. However, it has now being strongly suggested that early dissemination of tumor cell may produce micrometastasis and thereby establish parallel progression model of cancer [Marusyk A et al., 2012; Gray JW, 2003]. The infiltrating tumor cells undergo Epithelial-Mesenchymal Transition (EMT), lose its proliferative capacity but retain its migratory ability to enter into the systemic blood circulation (Figure 1.17) [Christiansen JJ et al., 2006]. But majority of this dormant CTC population (85%) undergoes apoptosis and disappeared from blood circulation within few minutes [Glinsky VV et al., 2003; Berezovskaya O et al., 2005]. However, only a small fraction of the CTC population may invade blood vessel to enter in distant organs or in bone marrow as Disseminated Tumor Cells (DTC) and establish micrometastatic loci (Figure 1.17). The cells of such micrometastatic lesions may further undergo Mesenchymal-Epithelial Transition (MET) to gain its proliferative function and eventually generate macrometastatic lesions or parallel tumor growth (Figure 1.17) [Yang J et al., 2006; Christiansen JJ et al., 2006].

In parallel progression of tumor, the genetic divergence between primary and metastatic tumors brings in considerable heterogeneities, making the primary tumor difficult for therapeutic interventions [Marusyk A et al., 2012].

1.10.2 HR HPV in Circulating Tumor Cells (CTC): implications in CaCx prognosis

The importance of CTC, because of its association with tumor metastasis, has been implicated in disease prognosis. The mutation and methylation of different host genes and alterations of microsatellites markers were studied in
CTC of different types of cancer, mentioning its importance in disease prognosis [Mulcahy et al., 1998; Sorenson, 2000; Coulet et al., 2000; Gonzalez et al., 2000; Wong et al., 2000]. In CaCx patients, prevalence of HPV in plasma, before and after therapy has been suggested to be important for disease prognosis [Pornthanakasem et al., 2001; Singh et al., 2006]. Moreover, existence of integrated HPV DNA in plasma of primary CaCx patients was also reported [Pornthanakasem et al., 2001]. However, the genetic and epigenetic profile of HR HPV in pre and post therapy plasma of same CaCx patients will help understand its importance in early diagnosis and prognosis of the disease.
C. **Scope of the study**

Cervical Cancer (CaCx) is the second most common gynecological cancer in India and highest global burden of CaCx resides in India. Despite the fact, large scale studies on the population prevalence of Human Papillomavirus (HPV), the necessary causative agent of CaCx, from our country is limited. The average prevalence of cervical HPV infection in Indian population is 7.9% which is in concordance to the global prevalence of 10%, although, the frequency varied between 7-14% from region to region [as discussed in section 1.6]. Moreover, the distribution of High Risk HPV (HR HPV) types showed extreme variations among these studies. The diversity in ethnicity, geography, socio-cultural practices, size of the study population and methodologies used for HPV detection could be accountable for such variations. To reduce the CaCx incidence in India, the prevention of HR HPV infection is essential. But without exact knowledge on the prevalence rate and type distribution of HR HPV in the respective population, prevention of viral infection by the prophylactic vaccines cannot be successful, as the vaccines are type specific.

Nearly 80% of the cervical HPV infection is cleared off by host immunity, leaving ~20% women susceptible to develop different grades of cervical abnormalities due to persistent latent infection. Majority of these HPVs are HR HPV type 16 followed by HPV18 [as discussed in section 1.6]. It is reported that, the inefficient viral antigen presentation by the polymorphic HLA Class II DQB1 allele to the T-Helper cell predisposes the women to CaCx development [as discussed in section 1.7.1]. Whether the association of polymorphic HLA allele with immune-clearance of the virus favors its persistence, is not known. On the other hand, the pro-inflammatory cytokines, especially IL-1β, induces inflammatory signal against persistent HR HPV infections. The development of CaCx has been associated with increased secretion of IL-1β which in turn linked to the -511 and/or -31 promoter polymorphisms [as discussed in section 1.7.2]. However, the association of HLAII DQB1 and IL-1β -511 polymorphisms with the viral persistence, if any, has not yet been studied in detail.
Continual expression of HPV16 oncoproteins, particularly E6 and E7, is necessary for transformation of the cervical epithelium [as discussed in section 1.2]. It is evident that the expression of E6 and E7 depends on the genetic (physical status, viral copy number, sequence variation of the viral genome) and epigenetic (methylation of enhancer and the early-late promoters) profile of HPV16 genome [as discussed in section 1.9.1 & 1.9.2]. However, the genetic and epigenetic profiles of the virus in asymptomatic infection are not well studied. Moreover, the change of the profiles, if any, during development of the tumor from asymptomatic infection to pre-neoplastic lesions to CaCx is not well understood. Similarly, how these profiles of HPV16 change due to therapeutic interventions has not also been evaluated in details for better prognosis of the disease.

On the other hand, presence of HPV16 in plasma of both pre and post-therapy CaCx patients, even in cervical swabs of post-therapy patients has been detected [as discussed in section 1.9.1B and 1.10.2]. The importance of HPV16 presence in plasma has been associated with early diagnosis and prognosis of the disease [as discussed in section 1.10.2]. Although, the comprehensive analysis of the genetic and epigenetic profile of HPV16 in the patients’ plasma before and after therapy and in the post-therapy cervical swabs, has not yet been evaluated in detail for early diagnosis and better prognosis of the disease.

Thus, to understand the importance of the genetic and epigenetic profiles of HR HPV, particularly HPV16, in the development of CaCx, as well as in prognosis of the disease, our study has been focused on the following aspects:

1. Analysis of prevalence and susceptibility to HR HPV infection in uterine cervix of normal women and CaCx patients from India.

3. Genetic and epigenetic profile of HPV16 in asymptomatic cervical infections to malignant transformation.

4. Genetic and epigenetic status of HPV16 in CaCx patients before and after therapy: prognostic implications.