Chapter 5

General Discussion
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The association of viruses as etiological cause of cancer in human has increased the epidemiological incidences of the disease. Such association has also elicited immense scientific interest to explore the pathogenecity of the virus as a preventable cause of cancer. Although, no cancer linked to infections develops without additional modifications within the host genome (as discussed in section ); alterations in the viral life cycle, pathogenecity and in its molecular profile are also involved. The association between Human Papillomavirus (HPV) and development of Cervical Cancer (CaCx) is a prominent example of such viral carcinogenesis. Attempts have been made to prevent the development of CaCx with the discovery of the prophylactic vaccines against High Risk Human Papillomaviruses (HR HPVs), especially HPV16 and HPV18. In India, however, we stand far behind to achieve successful control over the spreading of HPV and occurrence of CaCx. The major lacunae in this regard are the largely unknown prevalence pattern of HPV in our women population and the insufficiency of scientific studies on identification of the individuals who are genetically more susceptible to develop the malignancy, due to persistent viral infection. It is also not clear how the genetic and epigenetic profile of HR HPV16 changes during development of CaCx from initial asymptomatic infection of cervical epithelium. Also it is pertinent to analyze whether these profiles of HPV16 change after therapeutic interventions, as well as in Circulating Tumor Cells (CTC) prevailed in plasma at pre and post-treatment conditions. The knowledge on these profiles of HPV16 at different stages of the tumor development would lead us to understand the molecular status of the virus needed for CaCx development and prognosis of the disease.

It was evident from our accumulated data (as illustrated in Figure 5.1) that the prevalence of HPV infecting the cervical epithelium of women in general population was 7.2% (as discussed in chapter 2.2A.2). The prevalence gradually increased with the development of cervical abnormalities from 6% in asymptomatic to 71.4% in pre-neoplastic (LSIL/HSIL) to 98% in CaCx samples (Figure 5.1). Among the HR HPV type prevalence studied, the HPV16 showed a similar trend of increasing prevalence from 2% in asymptomatic to 26.6% in pre-neoplastic to 91% in CaCx samples (Figure 5.1). In contrary, the HPV18 did not show such high trend in prevalence (0.7% in asymptomatic to 3.8% in pre-neoplastic to 8% in CaCx).
Figure 5.1: An overview of changes in the HR HPV profile in CaCx development and disease prognosis.
(Figure 5.1). With the development of cervical abnormalities, significant gradual increase in HPV viral load was also seen (as discussed in chapter 2.2A.2.3). Moreover, the younger women (25-34 years) who were married at early age (<20 years) and having >3 parities were at more risk of acquiring cervical HPV infection, as observed in our multivariate demographic analysis (Table 2.5).

In the case control study for identification of the susceptible allele involved in persistence of the HR HPV16 and 18 in cervical epithelium, we have indentified the association of HLA-DQB1*03 (rs6457617) and IL-1β -511 (rs16944) loci with the same (as discussed in chapter 2.2B.2) (Figure 5.1). The A-allele containing genotypes (i.e. G/A and A/A) of HLA-DQB1*03 were significantly associated with CaCx, whereas, the T-allele containing genotypes (i.e. C/T and T/T) of IL-1β showed increased risk of pre-neoplastic and CaCx development, compared to HPV negative or, HPV-cleared normal women (Table 2.8 and 2.9). More importantly, the women who were homozygous or heterozygous at these two polymorphic loci together (i.e. HLA-DQB1*03 A-allele and IL-1β -511 T-allele), were increasingly susceptible for development of pre-neoplastic lesions or the tumor (Table 2.10). The polymorphic HLA allele might have contributed to inefficient clearance of the HR HPV infection in cervix (Figure 5.1). Whereas, during inflammatory response against persistent HR HPV infection, increased production of pro-inflammatory cytokine IL-1β, from the polymorphic T-allele, might have predisposed the HPV16/18 positive women to CaCx development by the cytokines’ ability to induce cellular transformation.

During persistence of the virus in cervix, cellular transformation is induced (Figure 5.1). Though additional genetic/epigenetic alterations of host genes are required for development of the tumor, the changes in genetic and epigenetic profile of HR HPV seems to facilitate the neoplastic events (as discussed in chapter 3.1). In asymptomatic infection and pre-neoplastic lesion, although the episomal form was prevalent, low frequency of HPV16 in integrated form was also noted (Figure 5.1). In CaCx, however, integration was the predominant form. Importantly, the early promoter (p97) was hypermethylated in episomal cases of asymptomatic and pre-neoplastic lesions, but comparatively hypomethylated in the corresponding integrated cases (Figure 5.1). It could be legitimate to infer that a few asymptomatic and pre-neoplastic samples harboring the integrated HPV16 were more virulent and could produce more oncoproteins (i.e. E6/E7) and induce the malignant transformation,
whereas, most of the episomal forms might be cleared off immunologically (as discussed in chapter 3.5). The increased transcription of HPV16 E6/E7 in CaCx samples from the hypomethylated p97, irrespective of the viral physical status, substantiates the necessity of the viral oncoproteins in transformed epithelium. On the other hand, the capsid proteins, L1 and L2 would be necessary during the productive infection of HPV, for which the virus must be in episomal form. Hence it was worthwhile when we observed less p670 methylation in episomal cases whereas, in integrated cases, it was comparatively more methylated (Figure 5.1). Interestingly, for suppression of the late gene transcription, increased methylation of p670 would have occurred in episomal CaCx cases (Figure 5.1). Thus, our findings indicate that the differential molecular profile of HR HPV16 is needed for gradual increase in expression of viral oncoproteins (E6/E7) and decrease in expression of capsid proteins (L1 and L2) during cervical carcinogenesis.

It was evident that the presence of HPV16 in the patients’ plasma was associated with higher viral-load in the primary tumor site (Figure 5.1). Thus, conversely, detection of the virus in plasma would indicate its status in the primary tumor. It has been seen that the HPV16 status in plasma could differ than the corresponding primary tumors in some patients (Table 4.2). This discordance might be due to differential migration of the tumor cells from the primary tumor sites, probably due to its heterogeneity or changes in the viral status during the course of migration. Interestingly, our findings showed poor prognosis of the patients having discordance in the viral profile between primary tumors and corresponding plasma (Figure 4.4 and Figure 5.1). Thus, this discordance in the HPV16 profile has importance in poor patient’s outcome.

It was evident that, after therapeutic interventions, the integrated form of HPV16 with increased hypomethylation of enhancer and late promoter prevailed in the cervical swabs, compared to the corresponding primary tumors (Table 4.2). Similar to the pre-therapy plasma, discordance in methylation status of HPV16 was seen in some patients. This indicates that the therapeutic intervention might provide selection of the integrated and hypomethylated form of HPV16. Interestingly, the patients having differential genetic and epigenetic profile between plasma and corresponding cervical swabs showed recurrence of the disease with metastases in distant organs.

Thus it can be concluded from our study that persistent HR HPV16 infection in susceptible
women and subsequent changes in genetic and epigenetic profile of the virus are the
determining step in viral carcinogenesis of uterine cervix. In addition, the knowledge on
genetic and epigenetic profile of HPV16 in plasma, before or after therapeutic interventions
has importance in prognosis of CaCx.