Chapter 6

Summary & Conclusion
Summary
The study focuses on two main factors associated with the pathogenesis of cervical cancer – HPV infection and genetic alterations. The study was carried out on pretreatment cervical cancer biopsies from a cohort of Indian women with locally advanced stage of the disease. The major highlights are as follows:

- Incidence of HPV in 270 cervical cancer biopsies as determined by 3 primer sets – GP5+/6+, MY09/11 and SPF1/2, was as high as 95% (257/270).

- Genotyping of the samples positive for 24 HPV types using GP5+/6+ primers (n=178), by Luminex bead array, showed most samples (168/178) to be positive for one or more HR-HPV types indicating a high association of cervical cancer with HR-HPV infection. Of the 24 HPV genotypes included in the HPV Genotyping Kit, infection by HPV 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 73 were identified. HPV16 and/or HPV18 infection was most common - 114 samples being positive for HPV16 alone and 6 samples for HPV18 alone. This was followed by dual infection by HPV16/18 (n=12) and 16/45 (n=9).

- Genotyping of the remaining 92 samples (which were negative using GP primers), showed 49 samples to be positive for HPV16 and none for HPV18. Therefore, the overall prevalence of HPV16 and/or HPV18 in this cohort was 72%, whereas overall HPV positivity was 95%.

- Patients positive for HPV16 alone showed a trend for better survival following radiotherapy as compared to HPV18 alone and dual infection by HPV16/18.

- Physical state of the virus as well site of viral integration was determined in a subset of cases (n=86) positive for HPV16 and/or HPV18 by APOT assay. Integration was observed in 79% cases (n =68), whereas in 21% (n =18) only episomal transcripts were identified. In 12 cases both integrated and episomal form of HPV were detected.
Comparison with clinical outcome revealed 16 out of 18 patients with only episomal form of HPV (16 and/or 18), to have disease free survival as compared to those with integrated form (p= 0.067).

The site of viral integration could be predicted with a high score in 48 cases and was found to be more frequent at chromosomal loci 1p (n =7), 3q (n= 8), 13q (n= 4), 6q (n =4), 11q (n= 4) and 20q (n= 4). Only one sample showed HPV integration at two chromosomal loci simultaneously.

Comparison with clinical outcome revealed that patients with integration at loci 1p (7/7), 6q (4/4) and 11q (4/4) were disease free, while most of them with integration at loci 3q (5/8), 13q (4/4) and 20q (2/4) showed recurrence of the disease in the form of either loco-regional or distant metastasis.

With NCBI Fragile site Map Viewer and UCSC Blat tool, most of the integration sites were found to be within or close to a fragile site (29/48) and/or protein coding genes (28/48).

Estimation of viral load in the same 86 samples by SYBR green based relative qRT-PCR, identified a wide range of viral titre across the samples, with the average being 47.5.

High viral load combined with viral integration showed poor prognosis; thus viral load and state of the virus can serve as a good prognostic marker of the disease.

Whole exome sequencing study of 11 cervical tumour and 8 matched control (blood from the same individual), in two sets (Dataset-I and II) identified a number of novel, nonsynonymous variations, that were validated by Sanger sequencing and/or customized Agilent SNP array. These might represent SNPs characteristic to the Indian genome and could add on to the existing dbSNP.
While germline variation of RNASEL, PTPRJ, ZFP64, FBN1, SREB1 and BANF1, identified from this data and validated by Sanger sequencing, might represent predisposing mutations to cervical cancer, missense mutations in FGF7, SOS2, DIDO1 and MAP3K3 as well as nonsense mutation in RASA1, might act as potential disease causing mutations.

Copy number alterations in chromosomal regions 1q, 3p, 3q, 8q, 11q, 17q and Xq were predicted.

Putative loss of heterozygosity in the chromosomal regions 2q, 3p and 11q was also predicted by ‘ExomeCNV’ package from the whole exome sequencing data.

Conclusion

The significance of the study lies in the fact that we have addressed all the major factors associated with pathogenesis of cervical cancer and assessed the role of each. The findings related to several viral cofactors – incidence, genotype, integration and viral load gives a detailed overview of the importance of each individually and also in relation to one another, in the context of cervical carcinogenesis. Through this study, we have showed for the first time, association between site of viral integration in the host genome and disease prognosis. Also, the combined effect of viral load and physical status on disease prognosis was demonstrated for the first time in the Indian cohort. The whole exome sequencing data from cervical cancer patients reported here, besides being a source of information on novel variations in the Indian genome, would mark the beginning of understanding the genomic landscape of cervical cancer.