Chapter 5

General Discussion

&

Study Perspective
Cancer of the cervix is estimated to affect approximately 530000 women each year worldwide with >88% of the burden being felt in the developing countries. In India it is the most common cancer as well as the major cause of cancer deaths among women (GLOBOCAN, 2008b; IARC, 2009). The disease is usually preceded by a well-defined and long pre-malignant phase, characterized by a series of microscopic events progressing from cellular atypia to various grades of dysplasia or cervical intraepithelial neoplasia (CIN) and eventually invasive carcinoma (Sellors and Sankaranarayanan, 2003). Persistent infection with high risk Human Papillomavirus (HR-HPV) is almost the universal etiological factor associated with the disease. The virus infects the dividing cells of the epithelial basal layer, continues to divide and proliferate in the upper epithelial layers, ultimately driving the cells towards malignant progression (Doorbar, 2006). The viral oncogenes - E6 and E7, are responsible for bringing about this transformation via interfering mainly with the cell cycle regulators p53 and Rb respectively. Several viral co-factors such as infecting HPV type, viral integration status and viral load determine the fate of virus-host interaction and play a key role in disease progression and predicting patient prognosis. Although, HPV infection plays a major role, it might not be sufficient for disease development (Cui et al., 2009). The evidence for this comes from the observation that not all HPV positive women develop the disease; also the window period between acquiring an infection and showing the symptoms is very long. All these hint towards a possible involvement of genetic factors in genesis of cervical cancer. Over the years, studies have reported role of SNPs, several point mutations, insertions, deletions and chromosomal abnormalities in cervical carcinogenesis.

Through this study we have attempted to address all the major aspects involved in the pathogenesis of cervical cancer such as HPV infection, including the role of various
viral cofactors such as genotype, physical state of the virus, site of viral integration and viral load; as well as, genetic alterations that might either contribute directly or predispose an individual to disease development. Such approaches would help in better understanding the fundamental rules of cervical carcinogenesis and be a step forward towards identifying biomarkers and newer treatment modalities for management and cure of the disease.

Role of HPV in cervical carcinoma

Reports from across the globe indicate a prevalence of HPV ranging from 73–99% in cervical carcinoma, with the highest incidences being reported from the Indian subcontinent (Basu et al., 2009; Bhatla et al., 2008; de Sanjose et al., 2010; Franceschi et al., 2003; Grace Nirmala and Narendhirakannan, 2012; Li et al., 2011). Of all the HPV types infection by HPV16 is most common, generally followed by HPV18, 31, 33 and 45 (Basu et al., 2011; de Sanjose et al., 2010; Grace Nirmala and Narendhirakannan, 2012; Li et al., 2011; Pillai et al., 2010). In the present study, the incidence of HPV infection (95%) as well as the prevalence of the two HR-HPV types – HPV 16 and/or 18 (72%) in the 270 pre-treatment cervical cancer biopsies from Indian women, with locally advanced stage of the disease and undergoing radiation therapy was in agreement with the published reports. The study, therefore, might add to the existing reports on HPV detection and genotyping from the Indian subcontinent and contribute to the projected prophylactic vaccine trials in the country.

Reports on prognostic value of HPV genotypes indicate a strong correlation between infecting HPV types and response of patients to radiation therapy (Wang et al., 2010). In the present study, clinical outcome of the patients infected with the two most common HR-HPV types HPV16 and/or 18 revealed that HPV16 positive cases showed a
trend towards better disease free survival following radiation therapy, as compared to HPV18 or dual infection by HPV16 and 18. However, this data was not statistically significant, probably owing to the small number of cases in the HPV18 positive group.

Apart from particular HPV genotypes, the physical status of the virus plays a major role in HPV induced cervical carcinogenesis. Although viral integration has long been associated with progression of the disease (Durst et al., 1985; Kalantari et al., 2001; Klaes et al., 1999), some recent reports have confirmed presence of episomal form alone in advanced cervical squamous cell carcinomas, thereby establishing that integration might not be absolutely mandatory for the process of carcinogenesis (Gray et al., 2010; Vinokurova et al., 2008). In the present study, only episomal form of HPV was detected in 18 out of 86 cases of advanced cervical carcinoma where the physical status of the virus was studied by APOT assay, further affirming these recent reports.

Reports regarding the significance of viral physical status in disease prognosis have been quite contradictory. While studies by Kalantari et al and Vernon et al. have demonstrated a decrease in disease free survival with viral integration (Kalantari et al., 1998; Vernon et al., 1997), according to some recent studies physical state of the virus bear no correlation with disease prognosis (Holm et al., 2008; Nambaru et al., 2009). In our study we found a significant association between viral physical status with disease outcome, the episomal form being associated with an increased disease free survival as compared to the integrated one.

Contrary to most of the earlier reports that considered integration to be a random event (Klaes et al., 1999; Vinokurova et al., 2008), our study identified certain chromosomal regions to be frequently affected by integration. Two recent reports have identified 3q28, 4q13.3, 8q24.21 and 13q22.1 as preferred sites for viral integration (Kraus et al., 2008; Schmitz et al., 2012) which support our findings. Our observation
that regions within or near known genes and chromosomal fragile sites might represent ‘hotspots’ for viral integration was in agreement with the published reports (Kraus et al., 2008; Matovina et al., 2009; Wentzensen et al., 2004). Comparison of the site of viral integration with clinical outcome revealed that not only the physical status of the virus, but also the site of integration is important in predicting the disease prognosis, since patients with integration in the chromosomal loci 3q, 13q and 20q showed an increased recurrence of the disease as compared to others. To the best of our knowledge this is the first report where the site of viral integration into the chromosomal loci has been shown to be an important factor in disease prognosis.

In order to have a clearer picture about disease prognosis, the study of viral integration was further complemented with estimation of viral load in the same 86 samples. Although several studies have identified viral load as an important marker for disease progression (Dalstein et al., 2003; Gravitt et al., 2007; Hernandez-Hernandez et al., 2003), none of these studies have taken into account the physical state of the virus. In the present study, association of viral load with survival of the patients as well as physical status revealed that, copy number of the virus combined with the physical state might serve as a good prognostic marker for the disease.

**Role of Genetic Alterations in cervical carcinoma**

Although mutations in important candidate genes such as Ras, PI3KA, STK11 (Cui et al., 2009; Madsen et al., 2008; Wingo et al., 2009; Wong et al., 1995) as well as several chromosomal aberrations (Narayan and Murty, 2010) have been associated with development of cervical carcinoma, till date there are no reports describing the genomic landscape of the disease. Therefore, in order to have a better understanding of the fundamental rules of the disease, whole exome sequencing of
DNA from 11 pre-treatment tumour biopsies (all of stage IIIB and >70% tumour) and 8 matched blood was done in 2 sets (dataset-I and II). Although initially SNP array was the method of choice for high throughput SNP analysis, with the availability of the far more developed and informative NGS technology at hand, we switched over to whole exome sequencing for the identification of the genetic alterations in cervical cancer. The sequencing data provided significant information about the novel variations in the Indian genome, the representation of which is inadequate in dbSNP. Therefore, our study could have immense contribution towards enriching the dbSNP with respect to the coding regions, in the Indian population.

Some of the germline variations in important candidates such as RNASEL, PTPRJ, ZFP64, FBN1 were identified from dataset-I and confirmed by Sanger sequencing, while somatic mutations in FGF7, MAP3K3, DIDO1, SOS2 and RASA1 were identified and confirmed from dataset-II. These might have significant implication in the context of carcinoma of the cervix. The germline variations could represent important predisposing mutations, while the somatic ones might directly be responsible for disease development. Studies have reported germline mutations of RNASEL including R462Q to be associated with prostate as well as cervical cancer susceptibility, (Barbisan et al., 2011; Casey et al., 2002; Madsen et al., 2008). Also, several missense mutations of FGF7, MAP3K3, RASA1 and SOS2 have been reported in the COSMIC database (http://www.sanger.ac.uk/genetics/CGP/cosmic/) for cancers of the breast, lung, kidney, ovary, prostrate, colon, etc. Besides, a recent study has identified exact identical mutation in DIDO1 (R303Q) in breast carcinoma (Stephens et al., 2012). To the best of our knowledge this is the first report stating the association of
mutations of these important proteins in cervical cancer. However, whether these mutations are actually ‘driver’ or ‘passenger’, would be really interesting and challenging to study.

The exome sequencing data was also used successfully to some extent to predict copy number variation and LOH that might be significant in cervical carcinogenesis. Overall, our study is the first demonstration of the application of whole exome sequencing in understanding the genomics of cervical carcinoma with special reference to the Indian population.

The overall work presented in the thesis is shown in Fig. 5.1.

Fig. 5.1 Overall summary of the study