ABSTRACT

DNA methylation is increasingly recognized as an important epigenetic event playing central role in regulating gene expression and aberrant methylation is shown to play central role in the initiation and progression of cancer in humans. Aberrant methylation in the form of loss (hypomethylation) or gain (hypermethylation) is commonly observed in cancer. Aberrant methylation of key genes is common in various cancers but is less well studied in cervical cancer in India. However, its impact on cervical carcinogenesis is not fully determined, and its potential as a diagnostic biomarker remains to be validated.

In the present study, an attempt has been made to understand the epigenetic aspects of cervical cancer (i) by evaluation the DNA methylation at genome wide as well as at gene specific level followed by their validation in a panel of non malignant, pre malignant and malignant cervical samples (ii) to identify the influence of methylation on the expression of corresponding genes and (iii) whether this silencing could be reactivated by demethylation.

In the present study we have used nested PCR and direct sequencing approach for Human papilloma virus (HPV) genotyping. The genome wide 5 methyl cytosine content which reflects the global methylation level was estimated using reverse phase HPLC (RP-HPLC). The genome wide methylation changes in CpG islands were identified by methylation sensitive arbitrarily primed PCR (MS-AP-PCR) and differential methylation hybridization based microarray approach (DMH-Microarray). The microarray data was processed through extensive bioinformatic analysis to identify ontological process altered, gene to gene interaction and pathways analysis. The results of the study (differentially methylated regions) were validated in a panel of non malignant and malignant cervical samples by dimethyl sulphoxide polymerase chain reaction (MS-DMSO-PCR), combined bisulfite restriction analysis (COBRA) and bisulfite genomic sequencing. Further we have also identified the expressed CpG island sequence tags in cervical cancer cell lines namely SiHa by demethylation using 5-aza-2-deoxy cytidine treatment followed by hybridization onto UHN 12K CpG island microarray chip. Further, the methylation and its role in gene expression status was identified using demethylation and reverse transcriptase PCR (RT-PCR).
In the present study we have genotyped the prevalence of HPV in non-malignant, pre-malignant and malignant cervical samples by nested PCR approach using PGMY9/11 and GP5+/GP6+ primers. A total of 186 cervical specimens (83 non malignant, 15 pre malignant and 88 malignant) were used for HPV genotyping. HPV prevalence in non malignant, pre malignant and malignant samples was 20.48%, 60% and 75% respectively. Further, HPV16 was the most prevalent HPV strain in both non malignant, premalignant and malignant cases.

The 5-methyl cytosine content which reflects the global methylation level was estimated by using RP-HPLC. Sixty cervical biopsy samples (20 normal and 40 tumor samples) and 3 cervical cancer cell lines namely SiHa, CaSki and HeLa were used for global methylation profiling. There was a significant reduction in global methylation content between normal and tumor samples. Further, the relationship between hypomethylation and cervical cancer was found to be statistically significant with “p” value 0.002 and t value 2.757 with degrees of freedom 52. Moreover it was also found that HPV positive samples were globally hypomethylated than the HPV negative samples and found to be statistically significant with a $p$ value $\leq 0.0020$.

In order to find the differentially methylated regions in cervical cancer at genome wide level we have used methylation sensitive arbitrarily primed polymerase chain reaction (MS-AP-PCR) and differential methylation hybridization (DMH) based microarray technique in combination with computational approach. The results were validated by combined bisulfite restriction analysis (COBRA) and bisulfite genomic sequencing (BGS). Further 5 genes namely DOC2B, DAPK1, RAB6C, CDKN2A and ZNF471 were selected for validated in a panel of non malignant and malignant cervical samples by COBRA and BGS. Further DOC2B, DAPK1, RAB6C, and ZNF471 were found to be frequently methylated in cervical cancer. Out of the five genes analyzed DOC2B, RAB6C and ZNF471 hypermethylation was reported for the first time in cervical cancer while hypermethylation of DOC2B and ZNF471 for the first time in any disease. Further, we have also shown evidence for the role of DNA hypermethylation and subsequent gene silencing of DOC2B and ZNF471 in cervical cancer. The Diagnostic significance of identified methylation markers were also elucidated in the study.

We have used UHN 12K CpG island microarray platform in combination with aPRIME technique to develop a comprehensive approach for the identification of methylated genomic regions in cervical cancer cell lines namely SiHa and CaSki respectively. Additionally, we have
used the ECIST panel present in the UHN 12K CpG chip to identify the novel genes that are silenced via CpG island hypermethylation. The aPRIME approach resulted in the identification of several common and unique regions which are having differential methylation in SiHa and CaSki cells respectively. Further, we have also used extensive bioinformatic and system biology approach to unravel the ontological processes and biological pathways altered by methylation. In summary and future direction, the results are discussed in general based on the results of the studies presented in the thesis.

Results from our study reveals that aberrant DNA methylation might be one of the major cause of cancer in the studied cervical cancer population. Further, the study shows consequence of aberrant methylation in the loss of the expression of key regulator gene in cervical carcinogenesis. Our study further strengthens the knowledge of the pathogenic role of aberrant DNA methylation in cervical cancer. In the present study we have not only identified previously reported genes but also a number of genes whose functional role needs to be evaluated. Further the novel hypermethylated genes can be the potential candidate for further investigations to establish a possible relationship in aberrant methylation and cervical cancer. More over the study will be useful in molecular diagnosis of cervical cancer in India as well as worldwide. The observation from study can be used in early diagnosis, classification, prognosis and designing novel therapeutic strategies for cervical cancer and we recommend similar studies are required to support our result.