ABSTRACT

Cervical cancer (CaCx) is a multifactorial complex disease with a viral etiology of HPV infections. A large number of sexually active women incur HPV infections, only a fraction develop CaCx pointing to the involvement of both host and viral factors in disease pathogenesis. This study was aimed at understanding the roles played by some important viral as well as immunogenetic host factors in HPV16/18 related CaCx pathogenesis.

A case-control study was undertaken to test the hypothesis that cervical cancers (CaCx) harbor high HPV16 viral load compared to controls and this is influenced by E2 status and age of subjects. Viral load (natural log transformed values) per 100 ng genomic DNA was estimated [152 cases and 87 HPV16 (+)ve non-tumors] by Taqman assay. Median viral load was significantly higher (Mann–Whitney U test) among cases (17.21) compared to HPV16 (+)ve non-tumors (9.86), irrespective of E2 status or upon considering E2 status as a covariate in logistic regression model (p<0.001). Viral load of E2 intact cases (17.80) was significantly higher (p<0.001) compared to those with disrupted E2 (9.78). At equivalent probability of being a case, viral load was higher among individuals (i) of lower age, irrespective of E2 status, and (ii) with intact E2 but of similar age as those with disrupted E2. Thus viral load in association with E2 status and/or age might be of causal relevance in CaCx pathogenesis. This portion of the work has been published in Virology (2010) 354:197-202.

Since CaCx cases harboring intact HPV16 E2 gene portray a high viral load compared to those with E2 disruption, the next objective was to investigate whether these two forms of HPV16 genomes within CaCx cases are comparable or distinct in terms of (i) mRNA expression of the viral oncogene E7 and (ii) correlation if any between E7 mRNA expression and viral copy numbers. E7 and E2 mRNA expressions were quantified by real time PCR (RT-PCR) on a set of HPV16 +ve cases. All CaCx cases expressed E7 irrespective of E2 status, but E2 expression was recorded in E2 intact cases only. Viral load and E2 gene copy number were negatively correlated with E7C_{T}/β-actinC_{T} (p = 0.007) and E2C_{T}/β-actinC_{T} (p <0.0001) respectively, among E2-intact cases only. E7 expression in E2-intact cases was 75.087 folds higher (comparative C_{T} method based on 2^{-∆∆ C_{T}}) than of E2-disrupted cases. The findings provide novel insights into disease pathogenesis indicating that HPV16 positive CaCx cases harboring either intact or disrupted E2 gene are likely to be distinct at the molecular levels. This portion of the work has been communicated for publication consideration.

HLA class I genes are among the most polymorphic regions of human genome, with majority of the single nucleotide polymorphisms (SNP) occurring in the exons 2 and 3 that encode peptide binding cleft of the molecule. Based on resequencing of these
exons, in-depth analysis of single nucleotide polymorphisms (SNP) and haplotypes (reconstructed from significant SNP data) in HLA class I genes was determined. It has been possible to identify variations (SNPs/haplotypes) associated with either risk of or protection against CaCx pathogenesis. HLA class I allele-types were predicted considering IMGT/HLA Database based on large haplotypes reconstructed considering all SNPs (both biallelic and multiallelic; after initial quality control) which also depicted risk (B*4006010) and/or protective alleles (A*3303, A*0211, B*1502, B*3515). Median Joining NETWORK analysis identified some more such alleles and further revealed that haplotypes within the same HLA class I allele-type might differ in the extent of risk or protection. The small haplotypes reconstructed from significant SNPs (biallelic) imparting risk or protection could possibly be considered as signatures of associated risk or protection conferred by the large haplotypes defining specific HLA class I allele-types. Thus approach could thus serve as a novel method of identifying HLA allele disease associations with greater detection limit.

HLA class I mRNA expression level was estimated in various categories of cervical samples, HPV16 (+) cases compared to both HPV (-) controls and HPV16 (+) non-tumors. Significant downregulation of HLA A, B and C transcription was observed among CaCx cases both in contrast to HPV16 (+) ve non-tumors (4.78, 6.037 and 2.99 folds, respectively) and the HPV negative controls (8.38, 8.7 and 3.52 folds, respectively). Among the HPV16 positive CaCx cases, the E2 intact cases had 3.68 fold higher expression of HLA-A and 5.06 fold higher expression of HLA C, but no difference in HLA- B in comparison to E2-disrupted cases. Moreover, E2-disrupted cases, when compared to controls, showed more transcriptional repression (12.97, 15.62 and 11.49 folds respectively for HLA-A, HLA-B and HLA-C) than E2-intact cases, when compared to controls, (3.52, 3.99 and an insignificant 2.27 folds respectively for HLA-A, HLA-B and HLA-C). HLA class I promoter methylation was not found to be associated with cervical cancer pathogenesis.

This study is instrumental in providing insights into host-pathogen interaction in cervical cancer pathogenesis. It also highlights that these two types of HPV16 positive CaCx cases with intact or disrupted E2, are likely to be distinct biologically, thereby raising the issue of considering these forms of CaCx cases as separate entities in case control studies rather than classifying them into a single group. Furthermore, the study also offers unique approaches for molecular dissection of HPV viral genome status and genetic dissection of HLA diversity in a case control study.