Name of the Ph.D. scholar: Nisha Thakur

Supervisor: Prof. Seemi Farhat Basir

Co-supervisor: Dr. Mausumi Bharadwaj

Co-supervisor: Dr. B. C. Das

Department: Department of Biosciences, Faculty of Natural Sciences, Jamia Millia Islamia, New Delhi

Title: GENETIC ALTERATIONS IN CELL CYCLE REGULATORY GENES DURING THE DEVELOPMENT OF CERVICAL CANCER

Abstract:

Cervical cancer is the second most common cancer in women worldwide but a major cancer and a leading cause of cancer deaths among Indian women. India contributes to 25.4% and 26.5% of the global burden of cervical cancer cases and mortality, respectively. A large number of risk factors are known to contribute to high incidence of this disease but most important of them are early age of marriage, multiple sexual partners, multiple pregnancies, poor genital hygiene, smoking, use of contraceptives etc. But the most important factor has been considered to be is the infection with human papillomaviruses (HPVs). The primary cause in the development and progression of cervical neoplasia has been shown to be dependent on the integration of HPV DNA into the host genome. The two early genes of high-risk HPV types E6 and E7 encode the main transforming proteins. The products of these early genes interfere with the normal function of tumor suppressor genes p53 and Rb. So, with this back-ground, the present study is designed to see the gene alterations in p16 ^{INK4A}, cyclin D1 and RB1 in HPV associated cervical cancer in Indian women.

Present study showed that genetic variants in cell cycle regulatory genes (CCND1, p16, RB1) may collectively have potential to emerge as a biomarker for cervical cancer in Indian population. In conclusion, all the five studied SNPs in cell cycle regulatory genes are expected to have a substantial effect on the prediction models to distinguish women who are at the risk of developing cervical cancer.

Objectives of the study:

1. Detection of Human Papillomavirus infection in cervical carcinoma

2. Analysis of SNPs of p16 INK4A/Cyclin D1 (CCND1) and Retinoblastoma (RB1) genes in different grades of cervical carcinoma.

3. Identification of a panel of SNPs / haplotype as predisposition markers.

4. Evaluation of functional relevance of variant genes by expression and protein protein interaction studies in different cervical carcinoma cell lines in presence and absence of HPV as well as pRb.

5. Correlation of above findings with clinicopathological variables of the disease.

Methodology:

1. DNA Extraction and Quantitation 2. Polymerase Chain Reaction (β- globin, Detection of HPV, HPV Typing) 3. PCR-RFLP (Genotyping of CCND1/p16/RB1)

4. DNA Sequencing 5. Denaturing High Performance Liquid Chromatography (dHPLC) Analysis 6. Immunohistochemistry (IHC) 7. RNA Extraction and Quantitation 8. Reverse transcription-PCR (RT-PCR) 9. Functional assays

Major Findings:

In this case-control study about 85% of cervical cancer cases were found to be positive for HPV DNA sequence. However, only 4.5% healthy controls found to be HPV positive. CCND1 G870A, p16 C580T, RB1 A153104G single polymorphisms of cell cycle regulatory genes were found to be significantly associated with increased risk of cervical cancer. The significant linkage disequilibrium between CCND1 G/C1722 and G/A870 genotypes was observed in control group. CCND1 G1722C and p16 C540G polymorphisms may confer protection against the development of cervical cancer. P16 540C/580T haplotype was specifically found to be significantly associated with the risk of cervical cancer. Two SNPs were confirmed by dHPLC in cyclin D1: Functionally important SNP G870A (rs9344) in exon-4/intron boundary. SNP (rs5511137) in exon-1 of CCND1. Luciferase reporter assays and expression studies showed that the CCND1 3'UTR SNP rs678653 is of functional importance. Cyclin D1 protein expression was found to be associated with the frequency of CCND1 870AA genotype with the progression of cervical cancer. Cyclin D1 Transcript-a was over-expressed than Transcript-b in cervical cancer cases. Genetic variants in cell cycle regulatory genes (CCND1, p16, RB1) may collectively have potential to emerge as a biomarker for cervical cancer. This type of multigenic study alongwith haplotype analysis is more promising as compared to conventional single gene approach to establish the disease association.