Name of Research Scholar: Dr. Zakia Kazim
Name of Supervisor: Prof. Moshahid A Rizvi
Department: Deptt. Of Biosciences
Jamia Millia Islamia, New Delhi 110025.
Title of Thesis: Molecular Analysis of PTEN gene in Breast Cancer patients

Abstract

Background:

Cancer is medically neoplasm where cells divide and grow uncontrollably, forming malignant tumors, that invade nearby parts of the body. Cancer is the second most common cause of mortality in developed countries and epidemiologists predict in the coming year it will surpass the cardiovascular disease in terms of mortality. One of the most common malignancies in women is the breast cancer. Breast and ovarian cancer respectively are the 2nd and 5th leading cause of death among women. Breast cancer is a biologically and clinically heterogeneous disease where gene/pathway function changes by specific genetic and epigenetic alterations. Thus, understanding its growth potential and assessment of certain prognostic factors are of great importance in the prediction of the disease.

PTEN gene is considered an established tumor suppressor gene in different types of cancer including breast cancer. Inactivation of tumor suppressor can occur through the events like mutation, loss of one allele or Promoter methylation. Therefore the present study investigates the combinational frequencies of mutation, methylation, and loss of heterozygosity in the *PTEN* gene, especially in north Indian population along with their clinical parameters.

Methodology: 181 sporadic breast cancer and their adjacent normal tissues were included in the present study. Immunohistochemical methods were used to determine the expression of the *PTEN* gene in breast carcinoma, staining with an Anti-PTEN protein antibody was performed on formalin fixed paraffin embedded tissue. We also examined the expression pattern of receptors like ER,PR and Her2 by employing the same technique. We evaluated the methylation status of the CpG islands of PTEN using Methylation Specific PCR (MS-PCR) in sporadic breast carcinoma tissues and their adjacent normal tissues as control cases. The mutational analysis of *PTEN* gene was performed by Single Stranded Conformational Polymorphism (SSCP) briefly the PCR reaction was performed on isolated DNA from the fresh tissue samples and then reaction product was denatured and loaded onto polyacrylamide gel. After electrophoresis, the DNA was visualized by silver staining, on the samples where mutations were

found then automated DNA sequencing was performed. We evaluate allelic losses in microsatellites of the 10q23 region. The LOH analysis was performed by amplifying DNA through PCR, using three different markers of the 10q23 region (D10S541, D10S583 and D10S215). Assessment of LOH was based on visual comparison of the intensities of the normal DNA alleles and those of the tumors. All the molecular findings were correlated with the clinicopathological parameters of the patients to underline clinical relevance.

Results: We observed 54.1% (98/181) cases of LOH. Among 54.1% LOH cases 63.3% (62/98) were PTEN negative.We found 51.9% (94/181) PTEN promoter methylation. Among these 62.8% (59/94) cases exhibit loss of PTEN expression. We found that LOH and methylation of the *PTEN* promoter were significantly associated with loss of *PTEN* protein expression, while, *PTEN* mutation is a rare event, we found only 7% cases of mutation. Both LOH and *PTEN* promoter methylation were associated significantly with age, but their trends were different. Methylation and loss of *PTEN* expression correlated significantly with high grade and Her-2 negativity. On the other hand, LOH and methylation showed a significant relationship with clinical stage. We observed 53.5% (97/181) loss of PTEN protein. Furthermore, out of 32 double hit cases (*i.e.*, having both methylation and LOH), 87.5% (28/32) cases showed complete loss of *PTEN* expression (P= 0.0249). Interestingly, we observed a hormonal transition throughout the *PTEN* inactivation.

Our data suggest that methylation of the *PTEN* promoter plays prominent role in *PTEN* inactivation in a subset of breast carcinomas via biallelic epigenetic silencing while in another subset of breast carcinomas LOH is the predominant mechanism of *PTEN* silencing. Only in small amount of cases, a mixed genetic/epigenetic silencing (double hit) is evident with complete loss of *PTEN* expression. Hence, the uniformly suggested two structural hits would be the rule across a large array of tumors. However, in retrospect, it would appear that this sort of *PTEN* defect is not common in Indian breast cancer. Consequently, both LOH and methylation profile seems to be an important factor in predicting the clinical outcome of breast cancer patients

Conclusion: The pattern of *PTEN* expression and its correlation with the clinical parameters indicates that the Indian breast cancer patients have a hugely varied course of regulation of *PTEN* expression, where promoter methylation and LOH seem to play a greater role while PTEN mutation otherwise a major event has a pretty small contribution.