Abstract: Molecular Studies on *p16* Gene in Indian Female Breast Cancer Patients

A tumor-suppressor protein known as p16 usually suppresses the phosphorylation of retinoblastoma (RB) protein, a negative regulatory factor of the cell cycle at the phase of G1/S, and contributes to the inhibition of cell growth. On the contrary, inactivation of *p16* gene is considered to be involved in tumorigenesis by promoting cell growth. The inactivation of the *p16* gene is brought about by its homozygous deletion/LOH, point mutation, or the methylation of CpG islands in the promoter region, but the mechanisms differ with the cancer types. To find out the involvement of *p16* gene, we investigated the aberrant promoter hypermethylation and loss of heterozygosity (LOH) of *p16* gene in n=116 cases of Indian bresat cancer patients. The frequency of *p16* gene hypermethylation was 55.1% and the frequency of LOH at chromosome 9p21–12 was 85.3%. The frequency of two-hit inactivation of the *p16* gene by hypermethylation and LOH was 39.6%. Two-hit inactivation of the *p16* gene showed loss of protein expression and was significantly correlated with the different clinical stages of breast cancer. Moreover, we found a significant correlation between 5'CpG island methylation of *p16* gene and the CT genotype of *DNMT3b*. This finding suggest that CT genotype of *DNMT3b* gene has a low penetrance genetic risk factor and cause cancerous phenotype by aberrant gene inactivation of *p16* gene. Additionally, to find out the underlying mechanism of LOH, we tried to correlate the LOH of these four markers (D9S171, D9S200, D9S126 and D9S156) and the 5'CpG island methylation of potent DNA mismatch repair genes *hmlh1* and *hmsh2*. Herein, P value (P =0.01) suggest that LOH at *p16* gene solely occur due to the epigenetic silencing of *hmlh1* and *hmsh2* genes. A highly significant association was observed between LOH (+), hypermethylation (MM) and loss of p16 expression ($P = <0.0001$), suggesting that deletions and 5'CpG island methylation may represent an important mode of *CDKN2/p16*^{ink4a} gene inactivation. In conclusion, biallelic inactivation of the *p16* gene by hypermethylation and LOH might cause loss of p16 expression and play an important role in the development of breast cancer. Therefore, controlling and monitoring for hypermethylation and LOH of *p16* gene may be partially useful for the treatment and early diagnosis of early breast cancer.