Thesis Title: Methylation mediated gene silencing and polymorphism analysis of GSTP1 gene in Indian breast cancer patients

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ABSTRACT

Cancer is a leading cause of death worldwide. The main types of cancer leading to overall cancer mortality each year are lung, stomach, liver, colon and breast. Breast cancer is the most common cancer and the second most common cause of death from cancer in women. The highest incidence rates observed for breast cancer in India was about 32 per 100,000 females.

Further insight into the aetiology of breast cancer may be gained by identifying susceptibility factors that predispose individuals to breast cancer if they are exposed to particular environmental agents. An example of such candidate susceptibility factors would include inherited differences in carcinogen metabolism as observed for glutathione S-transferases (GSTs). The GSTs are a superfamily of genes whose gene products catalyze the conjugation of reactive chemical intermediates to soluble glutathione. GSTs are divided into four classes: GSTM1, GSTT1, GSTP1 and GSTA1.

GSTP1 is the most widely distributed and has been well studied in cancer. Two polymorphisms in the GSTP1 gene, one at codon 105 with an A to G transition and another at codon 114, have been reported. GSTM1 and GSTT1 also show polymorphism and homozygous null individuals lack a functional GSTM1 and GSTT1 protein respectively.

Hypermethylation of regulatory sequences at GSTP1 is associated with the loss of GSTP1 expression and has been found in the vast majority of human prostate carcinomas, renal and breast carcinoma.

In this polymorphism based case-control study, blood samples from a total of 823 subjects (413 breast cancer patients and 410 control) were collected from IRCH, All India Institute of Medical Sciences, New Delhi.

It was observed that patients who had menarche after the age of fifteen were at a slightly increased risk of breast cancer. Patients with history of smoking showed insignificant values but can be assumed to be at the threshold of breast cancer risk. Breast cancer risk was increased substantially in women with first-degree relatives with breast cancer.

GSTM1 gene was present in 192 patients and 269 control subjects. A total of 52.8% patients and 33.3% control subjects were found to have a deleted GSTM1 gene. No significant association was observed between breast cancer risk and GSTT1 null genotype. The prevalence of GSTP1 homozygous Ile/Ile was 36.2% in breast carcinoma and 49.6% in controls. The prevalence of Ile/Val was 47.5% in cases and 42.4% in controls. For the homozygous Val/Val, the prevalence was 16.3% in cases and 8% in controls. GSTM1 null genotype showed an almost two and a half fold increase in breast cancer risk. When dichotomised, it was observed that GSTM1 null genotype-related breast cancer risk was slightly decreased in post-menopausal women as compared to premenopausal group. A four times increase in breast cancer risk was observed in individuals who carry three high risk genotypes. Also, an almost three times more risk of developing breast cancer in individuals lacking GSTM1 and having at least one mutant allele in GSTP1.

For methylation and expression analyses, a total of 126 breast cancer tissue biopsies were collected from the operation theatre of IRCH, AIIMS. The promoter methylation was observed in a total of 34.1% samples. Statistically significant values were observed for locally advanced breast cancer cases and metastasized cases also showed relevance. In all, 36 out of 43 methylated samples showed absence of GSTP1 expression while the rest 7 showed varied levels of both nuclear and cytoplasmic GSTP1 expression. Out of 43 methylated samples, 90.7% samples showed no expression in western blot, while the remainder 9.3% showed varying degrees of expression.