MUTATIONAL AND EXPRESSION STUDIES OF *FHIT* GENE IN CERVICAL CANCER CASES

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INTRODUCTION

Cancer is a multigenic disease mostly somatic (Kinzler and Vogelstein, 1998; Lengauer et al., 1988). Cancer related genes fall into two categories: oncogenes that have a dominant effect and tumor suppressor genes with a recessive phenotype. Both classes of cancer genes have been identified through their alterations in human and animal cancer cells and by their elicitation of cancer phenotypes in experimental models (Bishop and Weinberg, 1996). Cervical cancer is one such cancer that affects more than five lakhs women worldwide each year. It is the eighth commonest cancer in the world and second most common cause of cancer related mortality among women worldwide.

Epidemiological studies reveal numbers of risk factors that contribute to the development of cervical cancer. These include infection with certain oncogenic type of human papillomavirus (HPVs), and other socio-economic factors (IARC, 1995; Bosch et al., 1995; Schiffman et al., 1996; Walboomers et al., 1999; Franco et al., 1999; Frenczy and Franco, 2002). Various studies indicate that HPV infection alone is not capable of transforming a normal epithelial cell to malignant one (zur Hausen 1994; Nishimura et al., 2000) hence additional genetic alterations seem to be required for the development and progression of cervical carcinoma (Mullokandov et al., 1996). Genetic abnormalities can take several forms such as point mutations, amplification or loss of heterozygosity. Furthermore, DNA methylation, which is a frequent epigenetic event, has been reported in many human cancers (Baylin et al., 1998; Costello et al., 2000).

Aberrant promoter methylation is an important mechanism for loss of gene function in tumors and may be more frequent than mutations in coding regions. Alterations of chromosome region 3p14 have been observed in numerous human malignancies. FHIT (fragile histidine triad) gene (spanning more than 1Mb) mapped to chromosome 3p14.2 have been reported to be involved in various human malignancies especially in epithelial tumors (Sozzi et al., 1996; Hayashi et al 1997; Zou et al 1997) including cervical cancer (Yoshino et al., 1988; Vecchione et al., 2001). FHIT as a candidate tumor suppressor gene, identified by positional cloning (Ohta et al., 1996). There are four 5' untranslated exons, three of which are centromeric to the 3p14.2 breakpoint and the remaining six exons are telomeric to this break point exon 10 is also untranslated exon 5-9 encode 1.1 kb mRNA that is translated into a 16.8 kDa protein, FHIT (Ohta et al., 1996). Although

FHIT clearly exhibits characteristics that differs from “classical” tumor suppressor gene, data from experiments typically used to identify and verify tumor suppressor gene provide the strongest evidence that FHIT encodes tumor suppressor.

To elucidate the role of FHIT gene in cervical cancer and its level of expression in different stages of tumors, the proposed study plans to undertake the analysis of the FHIT gene methylation which is a frequent epigenetic event in many human cancers, and expression by immunohistochemistry using anti FHIT antibody.

OBJECTIVES

- Study of mutational forms of FHIT gene in cervical cancer by SSCP method.
- Study of methylation status of FHIT gene by MSP (methylation specific PCR) method.
- Study of FHIT gene expression.

MATERIAL AND METHODS

Cervical cancer biopsies will be collected from different hospitals of Delhi. DNA isolation will be done by standard phenol chloroform extraction method. Mutational analysis will be done by PCR-SSCP, and methylation assay by MSP (methylation specific PCR) method. For immunohistochemical studies 5μ thick sections of the tumor biopsies will be obtained on Poly-L-lysine coated slides and expression will be studied by avidin-biotin method.

The study will show the correlation between methylation of the promoter region of FHIT gene and level of expression speculating the role of FHIT gene in cervical cancer, which could be important for both clinical as well as research point of view.