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RESEARCH

Allelic Loss of 10q23.3, the *PTEN* Gene Locus in Cervical Carcinoma from Northern Indian Population

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Abstract Cervical cancer is one of the most common malignant diseases affecting women worldwide. Studies on loss of heterozygosity have been made for *PTEN* gene specific microsatellite markers in malignancies like breast, ovary and lungs and the results have shown a significant association. However the role of this gene is not clearly understood in cervical cancer from Indian population. A total of 135 cervical carcinoma tissues samples were analyzed for loss of heterozygosity. DNA was isolated from the samples and their matched control specimens. Polymerase chain reaction was performed using primer specific for two intragenic markers (D10S198 & D10S192) and one marker (D10S541) in flanking region and further electrophoresed on 8% denaturing polyacrylamide gel. Overall, 31 out of 133 (23%) informative cases showed loss of heterozygosity in at least one locus in the region examined. The percentage of loss of heterozygosity for these markers ranged from 8% (D10S192) to 13% (D10S198). Loss of heterozygosity was more frequently detected in intragenic region (D10S198 & D10S192) than in flanking region, D10S541 (21% versus 9%). These data argue that *PTEN* is a tumor suppressor gene whose inactivation may play an important role in the carcinoma of uterine cervix.

Keywords Loss of heterozygosity · Squmaous cell carcinoma · Cervical carcinoma · Tumor suppressor gene

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Introduction

Tumorigenesis is the result of a multistep process resulting in genetic alterations that drive the progressive transformation of normal cells into malignant derivatives [1]. Frequent loss of heterozygosity (LOH) within genetically defined chromosomal regions is considered an indication of the presence of putative tumor suppressor genes [2, 3]. Several studies have shown that LOH at specific chromosomal sites is frequently associated with the development of various cancers [4, 5]. From several LOH studies, chromosomes 10q have been reported in the pathogenesis of a number of human malignancies, including lymphomas [6], gliomas [7], prostate [8], gastric and precancerous lesions [9].

Cervical cancer is one of the most common malignant diseases affecting women worldwide. India, which accounts for one sixth of the world's population, also bears one fifth of the world's burden of cervical cancer [10]. In India, cervical cancer is most common among woman-related cancers, followed by breast cancer, with an estimated 1, 32,082 new cases and 74,118 deaths [11]. In India, the number of deaths due to cervical cancer is estimated to rise to 79,000 by the year 2010. In urban areas, the incidence of cervical cancer is about 40% while in rural areas it accounts for 65% of cancers [12]. Epidemiologically, the main cause of cervical cancer is infection with certain types of human papilloma-

virus (HPV), a common sexually transmitted infection [13]. HPV types 16 and 18 have been reported to cause approximately 70% of all cervical cancers worldwide.

Loss of heterozygosity has been reported in cervical carcinoma on several chromosomal region including 3p, 6p, 10q, 11q, 17p [14–16]. A gene named *PTEN* (phosphatase and tensin homolog deleted on chromosome ten) or *MMAC1* (mutated in multiple advanced cancers 1) was isolated from the homozygous deletion region of chromosome 10q23.3 in several types of cancers or malignant cell lines [17, 18]. *PTEN* has been identified as a tumor-suppressor gene which negatively regulates the phosphatidylinositol 3-kinase (PI3K) signaling pathway [19]. *PTEN* downregulation was observed in many malignancies such as prostate cancer [20], neuroblastic tumors [21], endometrial tumors [22 and cervical carcinoma [23].LOH in *PTEN* gene locus has been frequently reported in cervical carcinoma [14–16, 24]. However, no such studies have been reported from Indian population.

Therefore, to define LOH on the long arm of Chromosome 10 and *PTEN* involvement in the progression of cervical carcinoma, we performed LOH using *PTEN* specific microsatellite markers which is localized within the *PTEN* gene or at flanking region of the *PTEN* gene in 135 paired samples from the squamous cell carcinoma of the uterine cervix.

Materials and Methods

Tissue Samples One hundred and thirty five cervical tissue biopsies of cervical carcinoma patients and their matched control samples (blood/precancerous lesions) were collected from Maulana Azad Medical College and associated LNJP Hospital, New Delhi and was immediately stored in −80°C. All histological diagnoses of cervical tumors were confirmed by gynecological pathologist. Tumors were staged according to the International Federation of Gynecologists and Obstetricians (FIGO) criteria [25]. Bioethics committee of the institution approved the study.

DNA Extraction DNA was extracted from the above frozen cervical tissue biopsies and their matched control samples by SDS/proteinase K treatment, phenol–chloroform extraction, and ethanol precipitation as described previously [26] and then dissolved and stored in TE buffer. Finally, purity and concentration of extracted DNA were analyzed by gel electrophoresis and ultraviolet spectrophotometry.

LOH Analysis Paired DNA samples from carcinoma tissues and matched blood were examined to detect loss of heterozygosity (LOH) with PCR based microsatellite analysis. Three microsatellite markers were chosen for this study. Markers D10S198 and D10S192 are within *PTEN* gene where as D10S541 is present in the flanking region. Primer sequences are available at the National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov/). PCR amplification was carried out in a final volume of 25 μL, containing 50 ng DNA, 2.5 μL of 10× PCR Buffer, 1.5 mmol/L $MgCl_2$, 0.5 μmol/L of each primer, 200 μmol/L of each dNTP, and 1 U Taq DNA polymerase. The amplification conditions were as follows: an initial incubation at 94°C for 10 min, followed by 35 cycles at 94°C for 30 s, at 58°C, 52°C and 57°C for 1 min for each of the above mentioned primers respectively, at 72°C for 1 min, and a final extension at 72°C for 10 min. The amplified products were denatured at 95°C for 5 min and rapidly cooled on ice. 5 μl of resulting products were run on 8% denaturing polyacrylamide gel(1xTBE buffer, circulatory water) at a voltage of 500 V, and power of 45 W for 3.5 h. Silver staining was performed as previously described [26].

Determination of PTEN LOH The heterozygous genomic allele was targeted for LOH information analysis. LOH was defined as a complete loss or up to 40% decreased relative

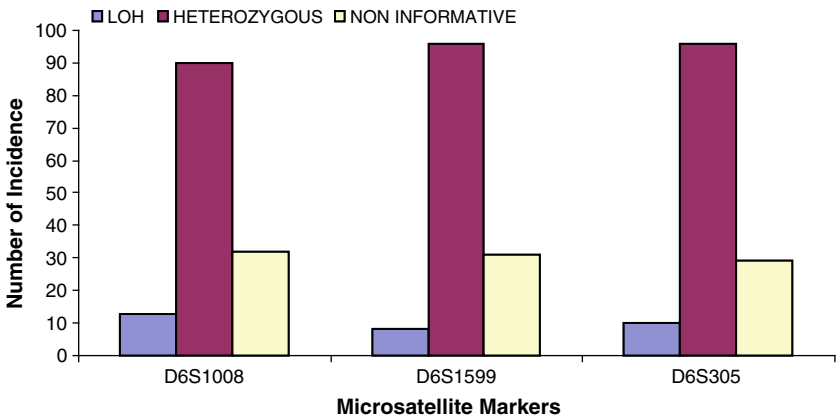

Fig. 1 Total incidence and frequency of LOH at 10q23.3 and *PTEN* gene locus

density of silver staining bands of PCR products in cervical cancer samples compared to their matched control samples [27].

Statistical Analysis LOH found in two intragenic markers (D10S198, D10S192) was compared with the marker at telomeric end (D10S541) using the *Chi-Square test* [28]. $P<0.05$ was considered statistically significant.

Result

We examined the allelic loss in 135 cervical carcinoma cases using three *PTEN* specific primer pairs located on the long arm f chromosome 10. A case is considered to be informative if the normal control tissue is heterozygous at that site. If the control is homozygous, it is not possible to detect LOH and is, therefore, uninformative.

Out of 135 cases examined, 133 were informative for at least one microsatellite markers. The percentage informative cases were 76, 77, 79 for the three different microsatellite markers; D10S198, D10S192, D10S541 respectively. The incidence and frequency of LOH for each primer pair is summarized in Fig. 1. In these informative cases 31 of 133 (23%) demonstrated LOH. Six of the patients showed LOH in both the intragenic markers (D10S198 & D10S192) and rest of the patients showed LOH in at least one of the loci examined. The percentage of LOH across each of the three markers ranged from 8% (D10S192) to 13% (D10S198). The highest rate of LOH was observed at intragenic marker D10S198 (13%) Overall, LOH was more frequently detected in intragenic region than in flanking region (21% versus 9%). Figure 2 shows an example of a silver stained gel depicting LOH and the normal control in the adjacent lane.

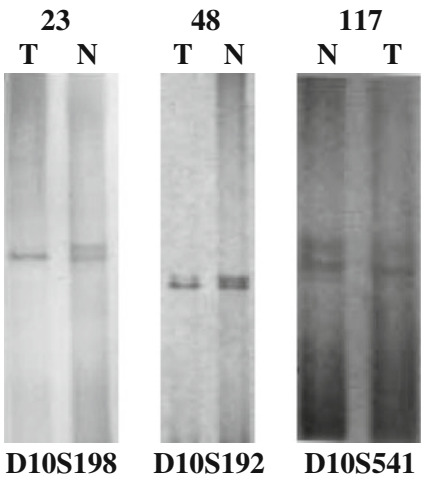

Fig. 2 Representative examples of microsatellite analysis of *PTEN* specific markers at 10q23.3. DNAs of tumor (T) and corresponding normal (N) tissues are shown with the microsatellite markers indicated at the bottom and sample numbers on the top

Correlation of Loss of Heterozygosity (LOH) at 10q23.3 with Clinicopathologic Characteristics

The clinical and pathologic parameters of 135 cases of cervical carcinoma are shown in Table 1. LOH on 10q23 of *PTEN* gene was observed in 4 of 44 (9%) patients below the age of 49 years whereas 27 out of 91 (30%) showed LOH at the age group of more than 50 years. The correlation of loss of heterozygosity was found to be statistically significant with the age ($P=0.007$). A significant association was also observed between LOH and clinical stage ($P=0.020$) as 3 out of 33 (9%) cases were found to be homozygously deleted in clinical stage I, 16 of 45 (35%) cases in stage II, 9 of 45 (20%) cases in stage III and 3 of 12 (25%) in stage IV. However, tumor grade was not found to be associated with loss of heterozygosity (LOH).

Discussion

Allelic imbalance by deletions or duplications of certain chromosomal regions is a major event in the development of various tumors. In this regard, LOH analysis has been

Table 1 Correlation between clinico-pathologic parameters and LOH at 10q23 (*PTEN* gene locus)

Clinicopathologic parameters	Total number cases tested ($n=135$)	LOH at 10q23.3		*P* value
		Positive (%) ($n=31$)	Negative ($n=104$)	
Age (years)				
≤50	44	04 (09)	40 (90)	0.007
>50	91	27 (30)	64 (70)	
Tumor grade				
G1	31	04 (13)	27 (87)	0.129
G2-G3	104	27 (26)	77 (74)	
Clinical stage				
I	33	03 (09)	30 (91)	0.020
II	45	16 (36)	29 (64)	
III-IV	57	12 (21)	45 (79)	
Histologic type				
SCC	126	29 (23)	97 (77)	0.956
AC	09	02 (20)	07 (78)	
Clinical Outcome				
Alive	74	35 (47)	39 (53)	0.056
DOC	61	19 (31)	42 (69)	

SCC Squamous cell carcinoma, *AC* Adenocarcinoma, *DOC* Died of Cancer.

found to be a useful tool in detecting deletions in the human genome [29]. LOH at the long arm of chromosome 10 constitutes an important role in the development of various cancers, including cervical carcinoma. LOH in *PTEN* gene locus has been frequently reported in cervical carcinoma [14–16, 24]. Here for the first time, allelic loss in *PTEN* gene locus is being reported in the carcinoma of human cervix from Indian population.

In this study, we proposed the region of analysis within *PTEN* gene locus and studied three microsatellite markers out of which two are present in the *PTEN* gene locus (D10S198 and D10S192) and one in the flanking region (D10S541) of the gene. The high rate of LOH was observed in the microsatellite marker D10S198 which is present within the *PTEN* gene locus. Therefore, this study prompted us to consider this gene as a strong tumor suppressor gene in the progression of the carcinoma of human cervix.

PTEN (also known as MMAC1 or TEP1) has been isolated from chromosome 10q23.3 [18, 30, 31]. The *PTEN* gene encodes a 403 amino acid protein homologous to some protein phosphatases, and the protein has been shown to possess protein phosphatase activity in vitro [32–34]. It is thought that *PTEN* protein dephosphorylates the 3 positions of phosphatidylinositol 3, 4, 5-triphosphate (PIP3), a well-known intracellular messenger of certain cell-growth stimulators [35, 36]. *PTEN* is found mutated in several cancer types that display LOH in this region [37, 38]. Previous studies have also reported LOH in 25–35% of cervical carcinomas, including squamous cell carcinoma and adenocarcinomas [14–16, 24]. LOH was also reported in 12.9% cases of total specimen within and flanking region of *PTEN* gene in SCC cases of uterine cervix [18]. Similar study showed LOH within or flanking the *PTEN* gene in 7 out of 19 (37%) cervical carcinoma and suggested bi-allelic structural *PTEN* defects may be necessary for cervical carcinogenesis [24]. Mitra et al., also evaluated the Indian patients for LOH at long arm of chromosome 10 and reported a frequency of LOH (28%) in cervical carcinoma cases [14]. These data suggest that LOH in *PTEN* gene is a common phenomenon in cervical carcinoma patients.

The present study supports the previous studies and suggests that *PTEN* is a putative tumor suppressor gene at human chromosome 10q23.3 and microsatellite analysis of this gene specific markers revealed that its reduced expression and inactivation may play an important role in the progression of cervical carcinoma and other human cancers.

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