J Biochem Tech (2012) 3(4): 328-330
ISSN: 0974-2328

Role of chromosome 3p22.3 in the development of cervical cancer

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Received: 09 March 2011 / Received in revised form: 14 March 2011, Accepted: 25 March 2011, Published online: 20 February 2012,

Abstract

The aim of this study was to exiamine the expression of chromosome 3p22.3 and its role in the development of cervical cancer. The 42 unrelated individuals freshly operated specimens from the cervical region with their corresponding normal or peripheral blood leucocytes (PBL) were collected from the patients. The specimens were undertaken for microdissection procedure. The detection of human papilloma virus (HPV) was done by PCR using the primers MY09 and MY11. The 3p22.3 region appeared to be associated with the development of cervical carcinoma because of significant increase of loss of heterozygosity frequency. The differential deletions in the chromosome 3p22.3 region show that this region plays an important role in the development of cervical cancer.

Keywords*:* chromosome 3p22.3, cervical cancer, human papilloma virus

Introduction

Cancer may result due to a combination of physical, chemical, biological and genetic insult to individual cells and can develop at any age (Minamoto 1999). Cervical cancer usually arises in the transitional zone between squamous and columnar cell epithelium (Lazo 1999). It's main etiological factor is the infection with high-risk forms of the human papilloma virus (HPV). Recent evidence suggests that a polymorphic variant of the tumor suppressor p53 (p53 Arg) may represent a risk factor for cervical carcinogenesis (Massimi 1997).

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HPV is the most prevalent sexually transmitted infection in the world, occurring in almost 75 % of sexually active women (Groopman 1999). HPV can infect basal epithelial cells of the skin or inner lining of tissues and are categorized as cutaneous or mucosal types. Cutaneous types of HPV are epidermitrophic and target the skin of the hands and feet whereas, mucosal types infect the lining of the mouth, throat, respiratory tract or anogenital epithelium (Zur 1994). Based on their association with cervical cancer and precursor lesions, HPVs can also be grouped as high-risk and low-risk types whereas they have been implicated in 99.7 % of cervical squamous cell cancer cases worldwide (Ponten 1995).

Over 90 percent of cervical carcinoma starts in the lining of the surface of cervix and are also called squamous cell carcinoma. Most scientists believe that cervical warts or pre-invasive cervical cancer may develop over a period of months or years after the cervix is infected with HPV (Roden 1997). Mild dysplasia or cervical intraepithelial neoplasia (CIN-1) or grade -1, can progress to moderate dysplasia (CIN-2), then to severe dysplasia and carcinoma *in situ* (CIN-3) and eventually to invasive carcinoma. Most physicians believe that about two-third of all cases of severe dysplasia will progress to invasive cancer if left untreated. Chemotherapy, radiation therapy and hormone therapy are being used for the treatment of the early-onset cervical cancer (Cox 1999). Treatment depends on the age of the women and on the preference of the patients and her doctor.

The aim of the current study was to study the chromosomal region of 3p22.3 and to narrow down putative TSG (define them) loci, within 0 – 0.5 Mb by using microsatellite markers, in order to reveal their role in the development of cervical cancer.

Materials and Methods

Sample Collection and Clinical Data

Freshly operated specimens from the cervical region of 42 unrelated individuals with their corresponding normal or peripheral blood leucocytes (PBL) were collected from the patients of Indian origin with the mean age of 47 years. The samples were frozen immediately after collection and stored at -80^{o} C until use.

Microdissection and DNA Isolation

The normal cells present as contaminant in the specimen were removed by microdissection procedure (Nawroz 1997). More than 50 serial sections (10-20 µm) were taken on glass slides using a cryostat. The representative 5 µm sections from different regions of the specimens (beginning, middle and end) were stained with hematoxyline and eosin for diagnosis as well as for marking of the dysplastic epithelium or tumor rich regions. Then, the marked regions were meticulously dissected by the microdissection procedure. Samples containing less than 60% dysplastic epithelial / tumor cells were not taken for further analysis.

DNA from the microdissected dysplasia / tumors and their corresponding normal or PBL was extracted by Proteinase-K digestion followed by phenol-chloroform extraction (Sambrook 1989) After DNA isolation and purification, the purity of isolated DNA is checked by taking A_{260nm} / A_{280nm} ratio, and it should be in the range of 1.5 to 2.

Microsatellite Markers

Two highly polymorphic microsatellite markers showing ≥ 70% informativeness in our sample were selected for this analysis.

Polymerase Chain Reaction Analysis

The Polymerase chain reaction (PCR) analysis was carried out in a 20 µl PCR mix as described by Nawroz et al. 1997 with slight modification.

*Interpretation of Loss of Heterozygosity (LOH) and Microsatellite Size Alteration (MA***)**

The LOHs were determined by densitometric scanning of the autoradiographs. For informative cases, allelic loss was scored if there was complete loss of one allele or if the relative band intensity of one allele was decreased at least 50% in the tumor, compared with the same allele in the corresponding normal control. The value was calculated as the ratio of the band intensities of the larger to the smaller alleles in the tumor DNA divided by the same ratio in the corresponding normal DNA sample. A LOH index of > 1.5 (loss of the smaller allele) or < 0.67 (loss of the larger allele) was corresponding to at least 50 % reduction in relative band intensities (Melamed 1997).

The MA was scored if one or both alleles at a given locus showed size variation, i.e. either expansion or contraction, in comparison with the same alleles in normal control DNA (Wistuba 1999). For calculation of LOHs, samples showing homozygosity and MAs only were not considered (Dillon 1997). The samples showing both LOH and MA at the same locus were considered for calculating both LOH and MA.

Detection of HPV-16 and HPV-18

The presence of HPV in the cervical lesions was detected by performing PCR using primers (MYO9 and MY11) from the consensus L1 region. Typing of HPV 16/18 in the L1 positive samples were done by means of PCR using specific primers from the E6 region of HPV-16 and the E7 region of HPV18. The PCR products were electrophoresed in 2% agarose gel with ethidium bromide, visualized under ultraviolet light and photographed. For the final confirmation of the HPV types, after gel electrophoresis, the PCR products were transferred onto a nylon membrane for southern hybridization with (^{32}P) labeled HPV type specific probes. DNA from the SiHa (for HPV – 16) and Hela (for HPV – 18) cell lines and the HPV type specific plasmids were used as positive controls.

Results

To localize the exact position of the candidate TSG, we made a detailed deletion mapping of the 3p22.3 region using two microsatellite markers i.e., D3S1561 and D3S1611. Among these, the D3S1561 (3p22.3) marker showed higher LOH% of61% (17/28) and MA% of24% (6/25). In the analysis there was no homozygous deletion but a rare bi-allelic alteration (MA-2, LMA) was shown to be prevalent in the D3S1611 loci, indicating the location of candidate's TSG in this region (Tables 1, 2 and 3). Thus, the gradual increase of LOHs/Mas during progression of the tumor high frequencies of MAs, the rare bi-allelic alteration in and around high LOHs regions and the loss of wild type chromosome 3 in the late stages of tumor development have suggested that such alterations might provide growth advantage to the tumors (Franco EL 1985).
Finally, he presence of HPV was detected by PCR, using primers from the consensus L 1 region. 90% (38/42) of the samples showed HPV infection. Out of the 38 HPV positive samples, 18 were typed for HPV 16/18 using type specific primers. Out of the 18 samples, 11 were positive for HPV-16 and 7 for HPV-18.

Table 1: Informativeness of microsatellite markers of chromosome 3

Marker	Locus	Informativeness No. (%)	
D3S1561	3p22.3	30/38	79
D2S1611	3p22.3	34/40	85

Discussion

In our present study, we attempted to delineate a deletion map of chromosome 3 in 42 cervical carcinoma lesions with different clinical and histological stages from an Indian patient population. We found that the short arm of chromosome 3 was preferentially deleted during the development of cervical carcinoma. The 3p22.3 region appeared to be associated with the development of cervical carcinoma because of a significant increase in LOH frequency.

The D3S1611 marker is intragenic to the mismatch repair gene MLH 1 (Dixon K 2004). However, we did not see a high frequency of MA in the cervical lesions studied. This indicates that the mutator phenotype might not be prevalent in the development of cervical carcinoma. Thus, the high frequency of allelic losses at the MLH 1 locus suggests that apart from mismatch repair mechanism, the deregulation of other possible functions of MLH 1 might be associated with the progression of tumor. However, the analysis of other genetic/epigenetic alterations of the MLH 1 gene in cervical lesions need to be studied in order to elucidate the underlying mechanism of inactivation of this gene. Adjacent to the MLH 1 locus another TSG DLC 1 was found to be located about 1 Mb towards the centromere. The involvement of TSG DLC 1 in the development of cervical carcinoma has not yet been studied.

The presence of rare bi-allelic alterations in and around the high LOH regions indicates that the LOH / MA in one allele might impose some selective pressure on the other allele for deletion or size alteration, which would result in a growth advantage for the tumor (Guo Z 2000). The occurrence of interstitial alterations and loss of normal copy of chromosome 3 in some samples suggest that these chromosomal alterations are needed for the progression of tumor.

Table 2: Allele status of the microsatellite markers of 3p22.3 - 24.1 in cervical cancer

Markers	D3S1561	D3S1611		
Locus	3p22.3	3p22.3		
Distance in Mb.	36.4	37		
No of Tumour samples			**Stage**	**HPV Status**
01	RH	RH	I B	---
02	RH	LMA	I B	18
03	LOH	LOH	I B	---
04	NI	MA 2	I B	---
05	NI	RH	I B	16
06	LMA	LOH	I B	16
07	RH	MA 2	I B	18
08	LOH	LMA	I B	16
09	H	RH	I B	18
10	LOH	RH	I B	16
11	MA 2	NI	I B	16
12	H	RH	I B	18
13	RH	RH	I B	+
14	RH	RH	I B	+
15	LOH	RH	I B	+
16	RH	LOH	I B	+
17	H	RH	I B	18
18	H	RH	I B	16
19	LOH	RH	II B	16
20	H	RH	II B	16
21	LOH	H	II B	+
22	LMA	LOH	II B	+
23	LOH	H	II B	+
24	LOH	RH	II B	+
25	LOH	H	II B	+
26	H	RH	II B	+
27	LOH	RH	II B	+
28	LOH	H	II B	+
29	NI	NI	III B	16
30	NI	RH	III B	16
31	H	RH	III B	16
32	LOH	RH	III B	---
33	RH	H	III B	+
34	MA 2	LMA	III B	+
35	RH	RH	III B	+
36	RH	LOH	III B	+
37	RH	H	III B	+
38	RH	RH	III B	+
39	H	RH	III B	+
40	LMA	MA 2	III B	18
41	LMA	LOH	IV	18
42	LOH	MA 1	IV A	+
LOH %	61 % (17/28)	30 % (9/30)		
MA %	24 % (6/25)	17.6 % (6/34)		

H- Heterozygosity; RH – Retention of Heterozygosity; LOH – Loss of Heterozygosity; LMA – Loss of one allele and size alteration of the other; MA 1 – Microsatellite size Alteration of one allele; MA 2 - Microsatellite size Alteration of both allele; NI – Non-informative; '-' – Negative for HPV; '+' – Positive for HPV with L 1 primer; 16 – Positive for HPV 16; 18 – Positive for HPV18.

The high incidence (90%) of HPV infection in these samples suggests that HPV infection is prerequisite for the development of cervical lesions. However, the absence of a significant correlation between HPV infection and the highly deleted regions indicates that HPV infection might have a casual association with the onset of cervical carcinoma by affecting normal proliferation, differentiation and apoptosis and thus resulting in the accumulation of more mutation in the infected cells.

Conclusion

Our studies have shown that the differential deletions seen in chromosome 3p22.3 in cervical lesions are necessary for the development of cervical carcinoma progression. The loss of function of TSGs located in these regions may have a sequential cumulative effect in the development of this tumor. Along with these deletions, other molecular changes in the chromosome, such as MA, bi-allelic alterations, interstitial alterations and loss of normal copy of chromosome 3p might play some role in tumor progression by imposing a selective pressure that provides for the tumor's growth advantage. (Hale 1991).

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