

RESEARCH COMMUNICATION

Genetic Instability in Cervical Cancer Detected by Arbitrarily Primed Polymerase Chain Reaction**Rujinee Paditaporn¹, Suda Riengrojpitak¹, Phaibul Punyarit², Sangdean Chindavijak³, Anant Karalak³, Songsak Petmitr^{4*}****Abstract**

The genetic instability in 54 Thai cervical cancer tissues were analyzed by Arbitrarily Primed Polymerase Chain Reaction (AP-PCR). The band alterations produced from 54 arbitrary primers were compared between the DNA fingerprinting from the patients and their corresponding normal cervical tissues. Results revealed 7 arbitrary primers provided DNA alteration patterns. Of these, an allelic loss in tumor DNA was found in DNA fingerprinting obtained from primers F-2 (64.8%), F-11 (68.5%), U-8 (51.9%), AE-3 (75.9%), AE-11 (53.7%), respectively. Moreover, DNA amplification was exhibited in patterns with primers B-12 (42.6%), J-16 (24.1%) and U-8 (70.4%). When genetic instability was investigated for associations with clinicopathological features, only the DNA amplified fragment with primer U-8 was significantly associated with stage II (P=0.030). Likewise, allelic loss amplified from arbitrary primer AE-3 showed significantly associate with age lower than 50 years old (P=0.003). Our findings suggest that the DNA alteration fragments produced from arbitrary primers of U-8 and AE-11 might be relevant to the pathogenesis of cervical cancer in Thai patients.

Key Words: Cervical cancer - arbitrarily-primed PCR - genetic instability - tumor stage

Asian Pacific J Cancer Prev, 8, 109-122

Introduction

Cervical cancer (CC) is the second most common cancer in women worldwide (Parkin, 2001). It has a relatively high prevalence in low socio-economic class populations. In Thailand, cancer of the cervix uteri is the most common gynecologic malignancy among women (Pongsaa and Jindawijak, 2003). It has become clear that human papilloma virus (HPV) infection is the most important cause of cervical cancer (zur Hausen, 2002). In addition, smoking, diet and immune status, and genetics factor may also be associated with the cervical cancer development (Baay et al., 2004).

It is currently accepted that conversion of normal cells to cervical cancer requires multiple genetic alterations such as activation of H- and N-ras oncogenes (Mammas et al., 2004), transcriptional inactivation of tumor suppressor genes such as p53, Bax, Bcl-2 and Mdm2 (Soufla et al., 2005). Recently, several studies have been extensively performed to determine the correlation between genomic instability and various human cancers in order to find out proper genetic markers for effective treatments (Arribas et al., 1997; de Juan et al., 1998).

Arbitrarily-primed polymerase chain reaction (AP-PCR) is a DNA fingerprinting technique based on polymerase chain reaction (PCR) amplification of random

fragments of genomic DNA with single short primers of arbitrary nucleotide sequence. It can be used to detect gene alterations occurring during tumorigenesis of unknown, as well as, known genes (Navarro and Jorcano, 1999). The semi-quantitative nature of AP-PCR in DNA fingerprinting has proven to be a promising technique for identifying novel gene alterations in many human cancers, including colorectal cancer (Malkhosyan et al., 1998), lung cancer (de Juan et al., 1999), breast cancer (Singh and Roy, 2001), hepatocellular carcinoma (Zhang et al., 2004) and cholangiocarcinoma (Chariyalertsak et al., 2005).

The purpose of this study was to investigate genetic instability in Thai cervical cancer by AP-PCR with 54 random primers and to determine the relationship between these genetic instability and the clinicopathological characteristics of the patients with cervical cancer.

Materials and Methods*Specimens*

Fresh cervical cancer tissues and their corresponding white blood cells were collected from 54 patients at the Department of Obstetrics and Gynecology II, the National Cancer Institute (NCI) of Thailand, Bangkok, Thailand, from July 2000 to December 2002. This study was approved by the Ethics Committee, the NCI, Bangkok,

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Thailand. The pathological diagnosis came from the final judgment by pathologists of the NCI and the Phramongkutklo College of Medicine, Bangkok. The histology of tumor types and stages were determined according to the WHO classification.

The cancerous tissues were embedded in Optimum Cutting Temperature (OCT) and cryostat tissue sections were performed (10 µm for DNA extraction and 3 µm for microscopic examination) for 50 pieces per case. The histological features of the first and the last section of each tissue block were confirmed by microscopic examination under hematoxylin-eosin staining.

DNA isolation

The tissue section from each cancerous case was washed by normal saline solution to clean out OCT for 3 times, then incubated in lysis buffer (10 mM Tris HCl, pH 8.0, 400 mM NaCl, 2 mM EDTA) and 50 ml of 10% SDS and 20 mg/ml proteinase K) in shaking incubator at 37°C overnight. Genomic DNA was extracted by phenol-chloroform methods. The DNA pellet was dissolved in TE buffer and kept at -20°C prior used.

The normal DNA was isolated from white blood cells (WBC) of normal counterpart cases using the same protocol as previously described.

Arbitrarily-primed polymerase chain reaction

The arbitrary primers were selected from a set of 1,200 primers (Operon, USA). Using the DNAsis program, the primers having 80% homology to sense strand of human 18S and 28S rDNAs, were excluded. Finally, the set of 54 primers was used in this experiment. These are B-12, D-15, F-02, F-11, G-14, H-08, J-16, L-01, M-07, M-19, N-20, O-15, O-19, Q-07, Q-09, S-03, S-10, S-13, U-08, Y-07, Y-19, AA-12, AA-14, AB-19, AD-10, AD-15, AE-03, AE-11, AG-11, AH-05, AI-11, AJ-03, AN-18, AO-05, AO-10, AO-16, AO-19, AP-15, AP-19, AR-09, AR-15, AT-11, AT-17, AU-01, AW-12, AX-11, BB-03, BB-13, BC-17, BC-19, BD-15, BE-12, BF-12, and BG-04.

Arbitrarily-primed polymerase chain reaction (AP-PCR) was performed in the DNA isolated from 54 cancerous and their corresponding white blood cells, with 54 arbitrary primers, based on the protocol of Williams et al (1990) with modification. DNA amplifications were carried out in a 25 ml reaction mixture containing PCR buffer (10 mM Tris-Cl, pH 9.0, 50 mM KCl), 200 µM of each dNTP, 2.5 mM MgCl₂, 1 unit of Taq DNA polymerase (Pharmacia Biotech, USA), 0.8 µM of each primer and 100 ng of genomic DNA. DNA amplification was performed in a Gene Amp PCR System 2400 for 45 cycles. Each cycle consisted of denaturation at 95°C for 1 min, annealing at 36°C for 1 min and extension at 72°C for 2 min. AP-PCR products were electrophoresed in 1.4% agarose gel. Alterations of PCR band pattern were observed in cancerous specimens compared to normal counterparts.

Statistical analysis

Statistical analysis was carried out using SPSS (version 13.0). Chi-square test was used to determine the correlation between DNA amplified patterns and the clinicopathological data of the patients. Statistical significance was accepted at P < 0.05.

Results

Genetic Instability in Thai cervical cancer by AP-PCR

DNA fingerprint of 54 patients with cervical cancer and their corresponding normal WBC were screened for genetic instability using 54 arbitrary primers (Figure 1). The results revealed that 7 of 54 arbitrary primers provided high percentage of bands alteration in cervical tumor DNA. The highest frequency of band loss was found in DNA fingerprinting amplified from AE-3 primer (75.9%) followed by F-11 (68.5%), F-2(64.8%), and AE-11(53.7%), respectively (Figure 2A). On the other hand, band amplification was found with the high frequency in DNA pattern obtained from primer B-12 (42.6%), and J-16 (24.1%), consequently (Figure 2B). Interestingly DNA fingerprint from U-8 patients provided both allelic loss (51.9%) and DNA amplification (70.4%).

Correlation between Genetic Instability and Clinicopathological Features

The correlations between genetic instability identified with the 7 arbitrary primers and clinicopathological features are shown in Table 1. It has been shown that patients with

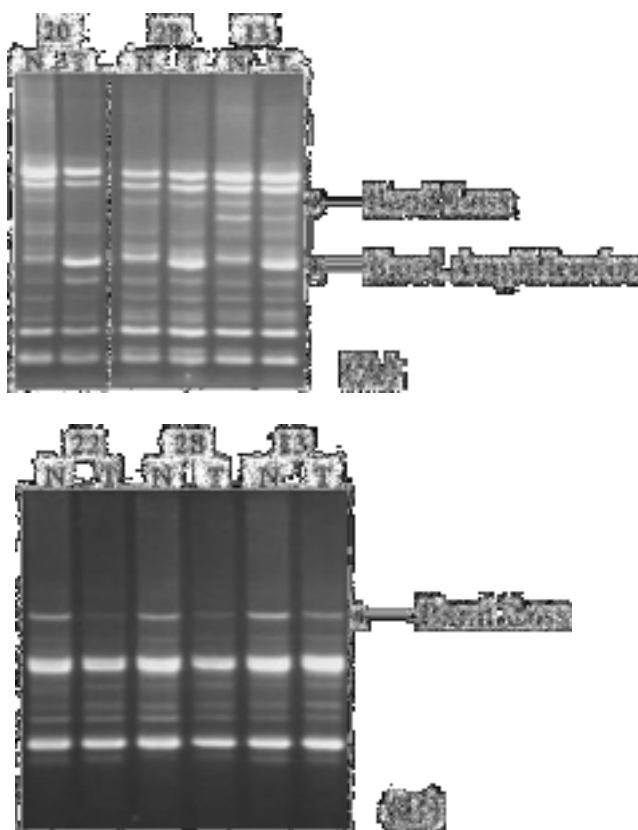


Figure 1. Representative AP-PCR Fingerprints of Cervical Cancer and Corresponding Normal WBCs Amplified with Arbitrary Primers U-8 (A) and AE-3 (B). The DNA fingerprints were electrophoresized on 1.4% agarose and visualized by ethidium bromide staining. There were alteration bands revealed in the fingerprints (arrow). (N = Normal DNA from WBC, T = Tumor DNA of cervix).

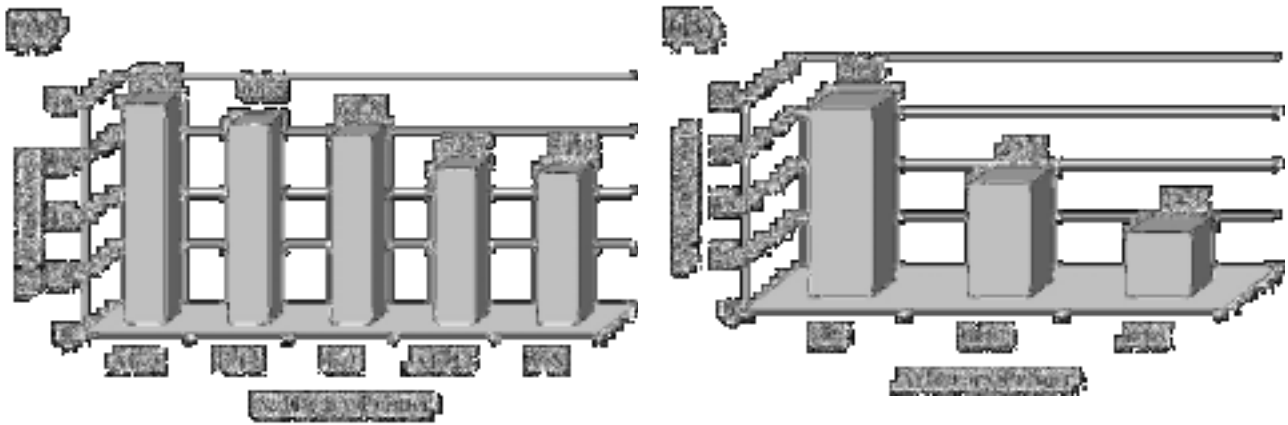


Figure 2. The Frequency of Genetic Instability in Thai Cervical Cancer assessed by AP-PCR. A) Illustrated the frequency of band loss by 5 arbitrary primers which are F-2, F-11, U-8, AE-3, and AE-11. B) Shows the frequency of band amplification by 3 arbitrary primers which are B-12, J-16 and U-8

cervical cancer stage II was significantly association with a DNA amplification in DNA fingerprinting amplified from arbitrary primer U-8 (Figure1A) compared to those with cervical cancer stage I and III (P=0.030). Moreover, The allelic loss in DNA pattern amplified from arbitrary primer AE-3 (Figure 1B) was correlated with the age of the patients less than 50 years old (P=0.003). There was no correlation between genetic instability and HPV infection status, histopathological types, and grading of the tumor.

Discussion

In human cancer cells several genomic regions are altered. The studies of genetic alterations such as gene amplification or gene loss have greatly facilitated by randomized screening with AP-PCR method. This method is a modification of PCR that generates a genomic DNA fingerprint using a single arbitrary primer (Navarro and Jorcano, 1999). The advantage of this method is that DNA alterations from unidentified chromosomal regions can be easily detected by comparison the DNA fingerprint between the tumor and the normal counterpart without

knowing the genome sequence. Therefore, AP-PCR techniques were required to allow screening for genetic markers of a human cancer (Arribas et al., 1997; de Juan et al., 1998). Ong and his colleagues (1998) studied genomic instability in 20 patients with lung cancer by AP-PCR using 7 arbitrary primers. They were able to detect genomic instability in 20 cancer tissues by each single primer ranged from 15 to 75%, and found that DNA changes were detected by at least one primer in 19 of 20 cancer tissues (95%). Whereas, Kawakami, et al (1998) and his colleagues used only one arbitrary primer to screens genomic alterations. They found specific loss of single intensity in the DNA fragment amplified from chromosome 10 about 34% in 15 tumors. Furthermore, DNA amplification in non small cell lung cancer (NSCLC) was detected by AP-PCR and found high frequency of gene gain (64% of all genomic changes analysis). The amplification intensity of the DNA fragment was also increased in 29(45%) of the 65 NSCLC patients (de Juan et al., 1999). In breast cancer, the researchers screened genomic instability by 30 arbitrary primers. They found only 9 (30%) arbitrary primers produced similar amplify PCR product patterns in all the DNA samples of breast tumors. Among these PCR product patterns, 65(34.2%)

Table 1. Correlation between Clinicopathological Features and Genetic Instability in Cervical Cancer

Parameters	U-8 Amp(+) n (%)	U-8 Amp(-) n (%)	P-value	U-8 Loss(+) n (%)	U-8Loss (-) n (%)	P-value	AE-3 Loss(+) n (%)	AE-3Loss (-) n (%)	P-value
Age (years)									
> 50	21 (70.0%)	9 (30.0%)	1.000	18 (60.0%)	12 (40.0%)	0.273	18 (60.0%)	12 (40.0%)	0.003*
< 50	17 (70.8%)	7 (29.2%)		10 (41.7%)	14 (58.3%)		23 (95.8%)	1 (4.2%)	
HPV infection									
Positive	30 (73.2%)	11 (26.8%)	0.493	23 (56.1%)	18 (43.9%)	0.346	30 (73.2%)	11 (26.8%)	0.485
Negative	8 (61.5%)	5 (38.5%)		5 (38.5%)	8 (61.5%)		11 (84.6%)	2 (15.4%)	
Histological type									
SCC	31 (73.8%)	11 (26.2%)	0.309	21 (50.0%)	21 (50.0%)	0.747	32 (76.2%)	10 (23.8%)	1.000
AdCA	7 (58.3%)	5 (41.7%)		7 (58.3%)	5 (41.7%)		9 (75.0%)	3 (25.0%)	
Grading									
Well diff	5 (62.5%)	3 (37.5%)	0.682	3 (37.5%)	5 (62.5%)	0.460	5 (62.5%)	3 (37.5%)	0.382
Other#	33 (71.7%)	13 (28.3%)		25 (54.4%)	21 (45.7%)		36 (78.3%)	10 (21.7%)	
Stage									
II	17 (89.5%)	2 (10.5%)	0.030*	11 (57.9%)	8 (42.1%)	0.577	16 (84.2%)	3 (15.8%)	0.341
I and III	21 (60.0%)	14 (40.0%)		17 (48.6%)	18 (51.4%)		25 (71.4%)	10 (28.6%)	

*Significant at the 0.05 level (2-tailed) #Moderately and poorly diferentiated

exhibited presence and absence or reductions and enhancements in the intensity in breast cancer tissues compared to uninvolved breast tissues from the same individuals (Singh and Roy, 2001). Shu-Hui et al (2004) studied genomic instability in hepatocellular carcinoma (HCC) by 10 arbitrary primers. All the cases of HCC were demonstrated the genomic instability by at least one primer. The incidence of genomic instability ranged from 20-70% in each case, and 17.9-50% in each primers. The clinicopathological features such as HBV infection, tumor size, histological grade, tumor capsule invasion were associated with the genomic instability on certain primers. From our reviews literature, this may the first report of the analysis of genomic instability in cervical cancer using AP-PCR techniques with 54 random primers. It worthy note that all random primers use in this experiment are not same as previously reported elsewhere. We observed all types of alteration provided by AP-PCR such as a allelic loss and/or DNA amplification in DNA fingerprinting occur with high frequency in cervical cancer; range from 24.07 to 75.93%. Interestingly, genetic alterations produced from primer U-8 was significantly association with stage II of cervical cancer.

In conclusion, our results revealed that the AP-PCR fingerprinting technique is an appropriate tool for the detection and analysis of genomic instability in cervical carcinogenesis thus providing a molecular alternative to the cancer carcinogenesis.

Acknowledgements

This study was supported in part by a UDC scholarship of Phramongkutklao College of Medicine, Faculty of Science, and the Faculty of Tropical Medicine, Mahidol University.

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