RESEARCH COMMUNICATION

Identification of Genetic Alterations in Thai Breast Cancer Patients by Arbitrarily Primed Polymerase Chain Reaction

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Abstract

Breast cancer is the most common cancer among women worldwide. Genetic alterations prevalent in breast cancer are still being elucidated. In this report, changes in 30 breast cancer tissues, in compariosn with normal tissues from Thai patients, were analyzed by arbitrarily primed polymerase chain reaction (AP-PCR). Genetic instability was detected by DNA fingerprinting obtained with 13 of 60 random primers. Of these, at least one amplification band, the incidence ranging from 27 to 80%, was observed in DNA amplified with 8 primers, whereas a band loss was exhibited with from 6 primers, the incidences ranging from 23 to 40%. Likewise, an amplification band amplified from primer D15 was observed in 80% of this patient group and a band loss produced from primer B12 presented in 40% of all cases. These results showed that AP-PCR is effective for the detection of genetic alterations in breast cancer tissues.

Key Words: Breast cancer - genetic alterations - arbitrarily primed PCR - random primer

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Introduction

The worldwide incidence of breast cancer is most common among women (Parkin et al., 2005). In Thailand, breast cancer is the second most frequent cancer in women (Martin et al., 2003). Genetic factors leading to the initiation, transformation and progression of normal cells turning into to malignant cells are the ultimate causes of all cancers (Skolnick et al., 1992). Two very important groups of genes, oncogenes and tumor suppressor genes, play key roles in determining whether a cell remains under normal controls or develops aberrant control signals and becomes a cancer cell (Hoffee, 1998).

The molecular basis of breast cancer comprises over-activation of proto-oncogenes and inactivation of tumor suppressor genes. Previous reports showed oncogene overexpression in approximately 50% of all breast cancers (Hubbard et al., 1994). The inactivation of tumor suppressor genes in breast cancer is involved with gene mutation. P53 is one of the most frequently mutated genes (20-40%) in sporadic breast cancer (Soussi et al., 1994). A simple new process, distinct from the PCR process, called arbitrarily primed polymerase chain reaction (AP-PCR), is based on the amplification of genomic DNA with a single primer of arbitrary nucleotide sequence. These primers detect polymorphisms in the absence of specific nucleotide sequence information and the polymorphism function as genetic markers and can be used to construct genetic maps (Williams et al., 1990; Hedrick et al., 1992). This technique is used extensively for detection of the rearrangement, addition, or deletion of DNA and ploidy changes in cells (Welsh and McClelland, 1990).

In this study, genetic alterations in the breast cancers of Thai patients were detected using AP-PCR technique with 60 random primers. The correlation between frequency of genetic alteration and the clinico-pathological parameters of the patients with breast cancer were also analyzed.

Materials and Methods

Specimens

Thirty breast cancer specimens, including normal tissues, were obtained from surgical operations. The fresh tissue samples were obtained from the Pathology Unit, Phramongkutklao Hospital, Bangkok. Breast epithelial cell histology was classified by hematoxylin and eosin-stain and observed under light microscopy. The clinico-pathological parameters involved in the breast cancer, routinely determined at the Pathology Unit of Phramongkutklao Hospital, were tumor size, histological grade, and status of axillary lymph node, estrogen receptor, and HER-2 expression.

DNA Isolation

Fresh samples were obtained and stored in a freezer at -70˚C until analysis. DNA from breast tumor tissue samples was extracted using proteinase K digestion and salting out (Miller et al., 1988). It was further quantified by measuring absorbance at 260 and 280 nm using a

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spectrophotometer, and a ratio of OD 260:280 at 1.5 to 1.8 was accepted for good quality DNA.

Selection of Arbitrary Primer

A set of 1,200 arbitrary primers (Operon, USA) was screened using the DNAsis program. Primers with nucleotide sequences having 80% homology to sense strand human 18S and 28S rDNAs were excluded. After screening, 60 arbitrary primers were selected for this study (A15, B12, D15, F2, F11, G14, H8, J3, J16, L1, M7, M19, N20, O15, O19, Q7, Q9, S3, S10, S13, U8, Y7, Y19, AA12, AA14, AB19, AD10, AD15, AE3, AE11, AG11, AH5, AJ3, AI11, AN18, AO5, AO10, AO16, AO19, AP15, AP19, AR9, AR15, AT11, AT17, AU1, AW12, AX11, AY19, AZ2, BB3, BB13, BC17, BC19, BD15, BE12, BF12, GB4, CO4, CO5).

Arbitrarily Primed Polymerase Chain Reaction

AP-PCR was performed based on the protocol of William et al. (1990). 25 ml of amplification mixture containing 100 ng of each genomic DNA from human breast cancer tumors and their corresponding normal tissues, 1X PCR buffer (10mM Tris-Cl, pH 9.0, 50mM KCl, 1.5 mM $MgCl_2$), 2.5mM $MgCl_2$, 200 mM each of dATP, dCTP, dGTP, dTTP, 1 unit of Taq DNA polymerase (Pharmacia Biotech, USA) and 0.4 mM arbitrary primer were used. Forty-five cycles of denaturation at 95˚C for 1 minute, primer annealing at 36˚C for 1 minute, and extension at 72˚C for 2 minutes, were performed in a programmed Gene Amp PCR System 9700. The AP-PCR product was mixed with loading buffer and loaded in 1.4% agarose gel and electrophoresed with 90 V for 1 h. The amplified products were visualized by ethidium bromide staining after resolving on gel under UV light. All tumors that presented an unstable genomic profile with change of band intensity (increase or decrease in signal intensity), band loss, band amplification when compared with their corresponding normal profiles, were defined.

Statistical Analysis

The correlation between frequency of genetic alteration and clinico-pathological parameters (tumor size, age, status of axillary lymph node, status of estrogen receptor, and status of HER-2 expression) were evaluated using Epi Info 6 software (CDC, USA), and P value < 0.05 was considered statistically significant.

Results

Genetic Instability in Breast Cancer

Sixty random primers were used to analyze 30 breast-cancer tissues and their corresponding normal tissues by AP-PCR technique, to detect genetic alterations in breast cancer (Figure 1). It was found that DNA fingerprinting amplified from 8 primers highly exhibited band amplification that ranged between 27-80% in each primer of the patient group (primers D15, F11, AB19, AE11, AO16, AY19, BB13, and BC17) (Figure 2); 6 primers highly exhibited band loss that ranged between 23-40% in each primer of the patient group (primers B12, Q9, S3, S10, AN18, and AO16) (Figure 3). The DNA fingerprinting

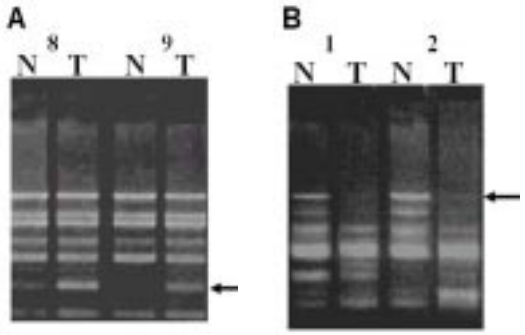

Figure 1. Determination of Genetic Instability in Breast Cancers by AP-PCR. Genomic DNA from breast cancer (T) and their normal counterparts (N) were amplified by AP-PCR and analyzed by electrophoresis on 1.4% agarose gel, then visualized by ethidium bromide staining. Panel A shows band amplification amplified from primer D15. Panel B presented band loss amplified from primer B12. Arrows indicate band amplification in panel A and band loss in panel B, respectively. Numbers represent the patient cases harboring genetic alterations

amplified from primer D15 showed the highest detectable band amplification in 24/30 samples (80%), and primer B12 showed the highest detectable band loss in 12/30 samples (40%). It is apparent that AP-PCR analysis may be useful for the detection of genetic instability in breast cancer.

Correlation of Genetic Instability with Clinico-pathological Parameters

The correlation between frequency of genetic alteration in each primer and clinico-pathological features in breast cancer were tested. No significant correlation was observed

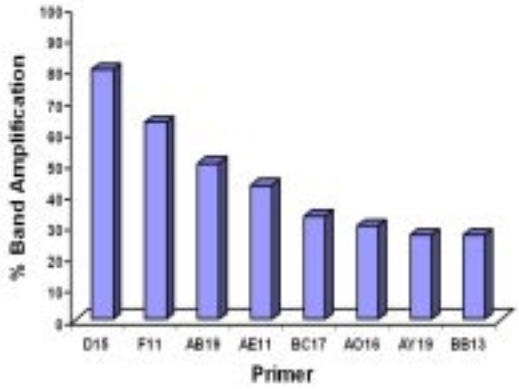

Figure 2. Percentage Band Amplification in Breast Cancers

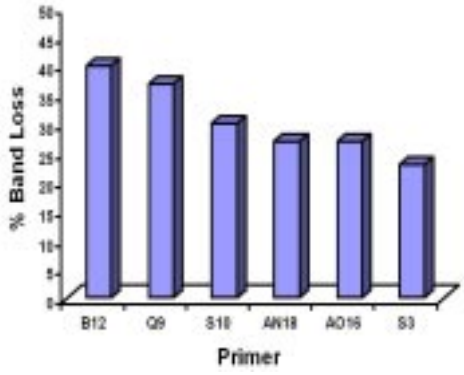

Figure 3. Percentage Band Loss in Breast Cancers

Table 1. Association between Band Amplification with Primer D15 and Clinico-pathological Parameters

Parameter		Number of cases		Odds ratio	P value
		Amp*	No amp	(95% CI)	
Age at diagnosis	≤50	10	3	1.20 (0.14-10.1)	0.60
	>50	12	3		
Tumor size (cm)	≤3	13	5	3.46 (0.29-92.6)	0.27
	>3	9	1		
Axillary nodes	+	12	4	0.60 (0.06-5.25)	0.47
	-	10	2		
ER status	+	9	1	3.21 (0.27-85.5)	0.30
	-	14	5		
HER-2	+	16	5	0.64 (0.02-8.85)	0.59
	-	5	1		
Tumor stage	1+2	17	5	1.76 (0.14-48.5)	0.54
	3	6	1		
Pathological grade	I+II	12	1	0.13 (0.00-1.75)	0.08
	III	6	4		

*Amplification

Table 2. Association between Band Amplification with Primer B12 and Clinico-pathological Parameters

Parameter		Number of cases		Odds ratio	P value
		Amp*	No amp	(95% CI)	
Age at diagnosis	≤50	7	6	0.21 (0.03-1.46)	0.07
	>50	3	12		
Tumor size (cm)	≤3	6	12	2.00 (0.32-13.15)	0.32
	>3	5	5		
Axillary nodes	+	5	11	0.64 (0.10-3.93)	0.43
	-	5	7		
ER status	+	2	8	0.28 (0.03-2.09)	0.15
	-	9	10		
HER-2	+	9	12	3.75 (0.31-100.9)	0.25
	-	1	5		
Tumor stage	1+2	9	13	1.08 (0.14-8.08)	0.63
	3	3	4		
Pathological grade	I+II	5	8	2.40 (0.33-18.7)	0.27
	III	6	4		

*Amplification

between the band amplification of primer D15 and band loss of primer B12 with age, status of axillary lymph node, status of estrogen receptor, Her-2 expression, tumor staging, tumor size, or histological grading of IDC (Tables 1 and 2).

Discussion

Different molecular methods have been developed to search for genetic alterations in cancer, such as deletion, amplification, and rearrangements of the tumor genome. AP-PCR was useful for the analysis of genetic alterations occurring during tumorigenesis (Scarpa et al., 1999). The advantages of AP-PCR for cancer studies are that it requires only small amounts of DNA, but does not require prior knowledge of any specific nucleotide sequence, and it is inexpensive (Navarro and Jorcano, 1999). The quantitative nature of AP-PCR fingerprinting also allows the detection of allelic loses and gains in tumor cells by reduction or increase in intensity of tumor fingerprint bands (Peinoado et al., 1992).

AP-PCR was widely used to study genetic alterations in cancer; e.g. Singh and Roy (2001) found that 34.2% of patients exhibited the presence and absence or reductions and enhancements in band intensity in breast-cancer tissues compared with uninvolved breast tissues from the same individuals. Maeda et al. (1999) reported genomic instability for each primer of 17-80% in head and neck cancers, whereas 17.9-50% genetic alterations amplified from each random primer were detected in hepatocellular carcinoma (Xian et al., 1999). In the present study, genetic alterations in breast cancer were identified using AP-PCR with 60 arbitrary primers; band amplification was 27-80% and band loss 23-40% in each primer. The important finding in this study is that a band amplification amplified from primer D15 was exhibited in 80% of breast cancer cases and a band loss produced from primer B12 in 40% of these patients. Moreover, genetic instability in breast cancer detected by gene amplification with primers D15 and B12 presented in 90% of this patient group.

A significant association between frequency of genomic instability identified by AP-PCR method and clinico-pathological parameters has been reported in many cancers; e.g. the frequency of genomic instability in early stage was significantly higher than the advanced stage in lung cancer (Anami et al., 2000), genetic alterations were more frequent in tumors < 3 cm in hepatocellular carcinoma (Xian et al., 2005), and high-grade breast cancer showed higher frequency of LOH than low-grade cancer (Piao et al., 2001). However, the genetic alterations in breast cancer detected from AP-PCR were not significantly associated with any clinico-pathological parameter, because the number of cancer tissues used in this study was relatively small. However, these results suggest that AP-PCR fingerprinting is an effective tool for identifying genetic instability in breast cancer.

In conclusion, genetic alterations in breast cancer from Thai patients could be observed from DNA fingerprinting obtained from AP-PCR with 60 random primers. A band amplification amplified from primer D15 was exhibited in 80%, and a band loss obtained from primer B12 presented in 40% of these patients, respectively. In addition, 90% of patients harbored either band amplification or band loss amplified from primers D15 and B12, respectively. These results reflect the advantage of the AP-PCR technique in determining genetic alterations in breast cancer.

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