Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Relationship between CYP1A1 polymorphisms and invasion and metastasis of breast cancer

Hua Wang, Wen-Jian Wang*

Laboratory of Department of Surgery, First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, Guangdong, People's Republic of China

ARTICLE INFO

Article history:
Received 10 June 2013
Received in revised form 15 July 2013
Accepted 15 August 2013
Available online 20 October 2013

Keywords:
Breast cancer
CytochromeP450
CYP1A1
Gene polymorphism
Invasion
Metastasis

ABSTRACT

Objective: To investigate the relationship between CYP1A1 genetic polymorphisms and the invasion and metastasis of breast cancer. **Methods:** The CYP1A1 gene polymorphism (an T-C transversion at nucleotide position 3801) was detected by the polymerase chain reaction and restriction fragment length polymorphism in 80 cases with breast cancer and 60 samples of normal breast tissue. The difference in genotypic distribution frequency between the groups, the correlation between the genotypes and the factors related to prognosis were analyzed. **Results:** The incidence of homozygous and variant genotypes had no difference between the breast cancer group and controls group (P=0.746). The proportion of variant genotype increased as clinical stage (P=0.006) advanced, as well as with increased numbers of lymph node metastases (P=0.010). **Conclusions:** In patients with breast cancer there is a correlation between the CYP1A1 CC allele and some factors indicating poor prognosis, including more lymph node metastases as well as a more advanced clinical stage.

1. Introduction

With the gradual development of the study on the genetic molecular biology techniques and human genomics, gene polymorphism is considered to be one of the important reasons for the differences of cancer between the individual. Cytochrome P450 1A1 (CYP1A1) as one of the most important phase Ⅰ metabolic detoxification enzymes *in vivo* has become the key enzyme involve in the metabolism of xenobiotic, its gene polymorphism has become the focus of the cancer research. In order to investigate the relationship between CYP1A1 genetic polymorphisms and the invasion and metastasis of breast cancer,the CYP1A1 gene polymorphism was detected in 80 cases with breast cancer and 60 samples of normal breast tissue.

*Corresponding authors: Wen-Jian Wang, Surgical Laboratory Department, the First Affiliated Hospital, Sun Yat-sen University, China.
Tel: 13316220070
E-mail: wh-hua225@163.com
Foundation project: It is supported by Guangzhou Municipal Science and Technology Support Program (No: 10A32060573).

2. Materials and methods

2.1. General information

Newly diagnosed patients with primary breast cancer were randomly selected from January 2012 to December 2012 in our hospital. A total of 80 cases served as the observation group, aged from 29 to 75 years, the average age was (48.6±16.3) years. All patients were in according to the diagnosis standard of breast cancer of the National Comprehensive Cancer Network guidelines, and did not receive chemotherapy. They had no family history of breast cancer or occupational exposure history of carcinogen. And 60 cases of healthy women who received normal physical examination in our hospital were selected as a normal control group at the same period, aged from 26 to 72 years, the average age was (49.9±14.7) years. The general information between the two groups showed no significant

difference (P> 0.05).

2.2. Main reagents and apparatus

PCR primers were synthesized by Shanghai Sangon Bioengineering Technology Service Co., Ltd., *Taq* DNA polymerase and restriction endonuclease were purchased from MBI. Blood genomic DNA extraction kits were purchased from the Beijing TIANGEN Technology Co., Ltd., the automatic gel imaging analysis device and multifunctional PCR instrument were purchased from Bio-Rad, USA. The multi-purpose electrophoresis tank was purchased from Shanghai KangHua Chemical and biological equipment company, high-speed freezing centrifuge was purchased from the MJ Research Inc company, USA.

2.3. Methods

Two mL of fasting venous blood were obtained with EDTA anticoagulant vacuum tubes, mixed by gently inverting and placed in the -80 ℃ refrigerator. DNA were extracted from all specimens according to the instructions of blood genomic DNA extraction kit, and then the CYP1A1 genotype was analyzed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. The CYP1A1 primers were: upstream, 5′-TAGGAGTCTTGTCTCATGCCT-3′, downstream, 5′-AGCGGCTACACCTCTTCACTG 3′. PCR reaction conditions were: 94 ℃ pre-denaturation for 5 min, then followed by cycles as follows: melted at 94 ℃ for 35 s, annealed at 60 ℃ for 35 s, extended at 72 ℃ for 60 s, after 33 cycles extended at 72 ℃ for 7 min again. Amplified fragment was 340 bp, PCR amplification product was digested with the restriction endonuclease MspI at 37 ℃ for 4 h. After 2% agarose gel electrophoresis,the photo are taken with PCR Gelation Image instrument and the digestion products were observed.

2.4. Determination

Bio-Rad image analysis system was adopted to observe the banding pattern of electrophoresis results and then photographed. The CYP1A1 gene 3801 (T/C) polymorphism was in the performance of three genotypes: TT wild homozygous type (340 bp), the TC mutations in heterozygous genotype (340 bp, 200 bp, 140 bp), CC homozygous genotype (200 bp, 140 bp).

2.5. Statistical analysis

All of the data were analyzed by SPSS16.0 statistics software. P<0.05 was considered as statistical significance. Hardy-Weinberg test was applied, P> 0.05 showed that the sampling may represent a genetic equilibrium population. χ^2 examination was used to test the genotype distribution frequency difference between the observation group and the control group, and the relationship between a variety of genotype and clinical features of breast cancer.

3. Results

3.1. CYP1A1 gene SNP-3801 (T / C) polymorphic loci RFLP typing results

PCR-RFLP method was used to determine the CYP1A1 gene SNP-3801 (T/C) polymorphism, if there was a T → C mutation in the sequence of the gene polymorphism of its PCR product, there would be three types after the restriction enzyme:

TT wild homozygous type (340 bp), the TC mutations in heterozygous genotype (340 bp, 200 bp, 140 bp), CC homozygous genotype (200 bp, 140 bp) (Figure 1).

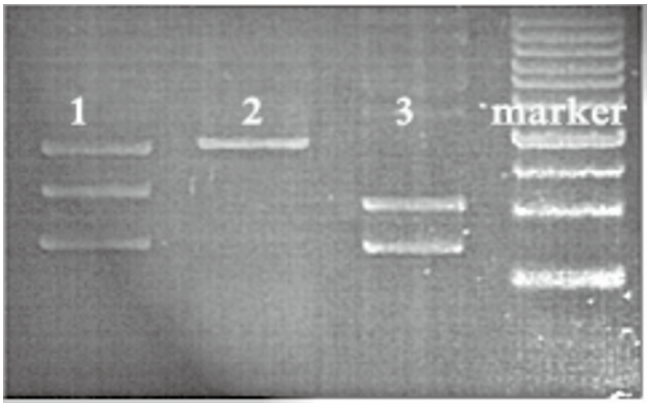

Figure 1. Three genotypes digested electrophoresis of two sites.
1: TT wild homozygous type (340 bp); 2: CC homozygous genotype (200 bp, 140 bp); 3: TC mutations in heterozygous genotype (340 bp, 200 bp, 140 bp).

In the observation group, the expected values of wild-type group, the heterozygous group and the homozygous mutations groups were 18, 50, 12, respectively. There was no statistically significant difference between them (χ^2=0.305, P=0.581). And in the control group, the expected values of wild-type group ,the heterozygous group and the homozygous mutations groups were 19, 27, 14, respectively. There was no statistically significant difference (χ^2=0.363, P=0.547), which showed the genotypes expected values and actual values were in good agreement between the observation group and control group. It indicated that it was accord with mendelian population genetics, and were able to represent the local female genetic equilibrium population (Table 1).

Table 1
Hardy-Weinberg equilibrium detection [n(%)].

Groups	Practical frequency			Theoretical frequency		
	TT	TC	CC	TT	TC	CC
Observation group (n=80)	21(26.3)	46(57.5)	13(16.2)	18(22.5)	50(62.5)	12(15.0)
Control group (n=60)	16(26.7)	33(55.0)	11(18.3)	19(31.7)	27(45.0)	14(23.3)

3.2. Frequency distribution of CYP1A1 gene 3801T→C genotype and allele in patients with breast cancer and in normal group

The frequency distribution of the TT wild homozygous type in the observation group and control group were 26.3% and 26.7%, respectively, and the frequency distribution of the TC mutations in heterozygous genotype in the observation group and control group were 57.5% and 55.0%, respectively. Frequency distribution of the CC homozygous genotype in the observation group and control group were 16.2% and 18.3%, respectively. There was no statistical significance difference in the genotype frequency distribution (χ^2=0.105, P=0. 746). The T allele frequency distribution of the observation group was 55.0%, in the control group was 54.2%, without significant difference (χ^2=0.019, P=0.890) (Table 2).

Table 2

Frequency distribution of CYP1A1 gene 3801T →C genotype and allele in the observation group and the control group [n(%)].

Groups	Genotype			Allele	
	TT	TC	CC	T	C
Observation group (n=80)	21(26.3)	46(57.5)	13(16.2)	88(55.0)	72(45.0)
Control group (n=60)	16(26.7)	33(55.0)	11(18.3)	65(54.2)	55(45.8)

3.3. Relationship between different CYP1A1 genotype breast cancers and prognostic factors

With the increase of the clinical stage (P=0.006) and the number of lymph node metastasis (P=0.010), the proportion of the variability genotype (CYP1A1-CC) become larger. There was no significant correlation between the CYP1A1 genotype and the age, tumor size and histological type of breast cancer patients (Table 3).

Table 3

Relationship of different CYP1A1 genotype breast cancers and prognostic factors.

Variable		n	TT	TC	CC	P value
Age (years)	≥50	18	6	10	2	0.502
	<50	62	15	36	11	
Size of the tumor	≥2	47	6	31	10	0.146
	<2	33	15	15	3	
Histological type	Invasive ductal	70	16	42	12	0.567
	Invasive lobular	3	2	1	0	
	Mucin cancer	2	1	1	0	
	Other factors	5	2	2	1	
TNM staging	Ⅰ	11	3	6	2	0.006
	Ⅱ A-Ⅱ B	54	15	34	5	
	Ⅲ	15	3	6	6	
Lymph node metastasis	Positive	25	5	12	8	0.010
	Negative	55	16	34	5	

4. Discussion

Breast cancer is one of common female malignant tumors. In 2011, the United States released the latest statistics which showed that 230 480 cases American women are expected to develop breast cancer reach in 2011. Its high incidence ranks the top of female cancer incidence rate. In China, the researches showed the incidence rate is also increasing. But in recent years, with the improvement of molecular biology techniques and polyclinics, breast cancer mortality has been declining. Pathogenesis of breast cancer is not yet clear, but there were some progress in the research of the development of breast cancer invasion and metastasishas. At the present stage, prevention of the invasion and metastasis of breast cancer is a key in the treatment of breast cancer. Breast cancer cells will depart from the primary lesion and then attach to the metastatic sites. Local infiltration, invasion and metastasis of the breast cancer are complex process of the joint participation of multiple genes.

It is considered that cytochrome P450 (cytochrome P450, CYP450) showed widely gene polymorphism, which can lead to obvious phenotypic polymorphism of individual differences and has a correlation with cancer susceptibility. Cytochrome P450 1A1 (cytochrome P450 1A1, CYP450 1A1) as endogenous compounds (hormones, fatty acids) and exogenous compounds (drugs, carcinogens, poisons) is an important member of the biotransformation enzymes and is closely related to the occurrence of tumor[1]. CYP4501A1 gene has 4 polymorphic sites, and CYP1A1*2A polymorphism is the 3′untranslated region, which is caused by mutations of 3801T→C (poly (A) sites downstream about 264 bp). There are three genotypes: TT-type (wild-type), TC (heterozygous-type) and CC (mutant-type), and all possess the Msp I identify restriction sites.

The CYP1A1 gene polymorphism was detected by PCR and restriction fragment length polymorphism in 80 cases with breast cancer and 60 samples of normal breast tissue. The result showed if there is a T→C mutation in the sequence of the gene polymorphism of its PCR product, there will be three types after the restriction enzyme: TT wild homozygous type (340 bp), TC mutations in heterozygous genotype (340 bp, 200 bp, 140 bp), CC homozygous genotype (200 bp, 140 bp). After Hardy-Weinberg equilibrium detection, our results showed that it is accord with mendelian population genetics, and were able to represent the local female genetic equilibrium population.

In recent years, studies confirmed that the susceptibility of cervical cancer, lung cancer, endometrial cancer, chronic myeloid leukemia and other malignancies may related with the gene polymorphism of cytochrome P4501A1. But some studies suggest that cytochrome P4501A1 gene polymorphism has nothing to do with the cancer incidence[2,3]. Zhang considered the mutant allele of the P4501A1 gene Ⅰ leVal polymorphic can significantly increase the risk of endometrial cancer[4]. Shi *et al* reports

that the gene polymorphism of CYP1A1 mSAP Ⅰ were related to the susceptibility of lung cancer, which may be associated with the gene mutation[5,6]. Zhu *et al* found that the occurrence of endometrial adenocarcinoma was related to the CYP1A1 gene exon 7 sub-Ile/Val genotype, which can be used as one of the indicators to screening endometrial adenocarcinoma susceptible population[7-9]. Jiang consider that the susceptibility of CML disease may be related to carrying CYP1A1C and the missing gene of GSTM1, T1[10,11].

The study on correlation of CYP1A1*2A polymorphic loci and the breast cancer showed that the distribution frequency of the TT genotype, TC genotype and CC type in the observation group were 26.3%, 57.5% and 16.2%, respectively, and in the control group were 26.7%, 55.0% and 18.3%, respectively. There was no statistical significance difference in genotype frequency distribution. T allele frequency distribution of the observation group and the control group were 55.0% and 54.2%, respectively; and C allele frequency distribution of the observation group and the control group were 45.0% and 45.8%, respectively, without significant difference.

The above results suggest that the gene frequency distribution of CYP1A1*2A polymorphism had no statistically significant difference in breast cancer, which is consistent with the report of Guo[12-15]. The results of the prognostic factors in patients with breast cancer and three genotypes of the CYP1A1*2A showed that the proportion of CC homozygous genotype will increase with the clinical stage of breast cancer, which was positively correlated to the increase of the number of lymph node metastasis in the process of cancer invasion and metastasis. There was no relevance between three genotypes and the age, the tumor size and histological type of the patients with breast cancer. These suggested that in the process of the invasion and metastasis of breast cancer, patients with CYP1A1*2A gene CC sites have a worse prognosis, so the clinical detection of CYP1A1*2A gene CC allele has prognostic value.

In summary, there is a correlation between the CYP1A1 CC allele and some factors, including lymph node metastases as well as clinical stage, which can significantly increase the risk of breast cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Reding KW, Chen C, Lowe K, Doody DR, Carlson CS, Chen CT, et al. Estrogen-related genes and their contribution to racial differences in breast cancer risk. *Cancer Causes Control* 2012; **23**(5): 671-681.

[2] Ashley-Martin J, VanLeeuwen J, Cribb A, Andreou P, Guernsey JR. Breast cancer risk, fungicide exposure and CYP1A1*2A gene-environment interactions in a province-wide case control study in Prince Edward Island, Canada. *Int J Environ Res Public Health* 2012; **9**(5): 1846-1858.

[3] Zhao YN, Zhang W, Chen YC, Fang F, Liu XQ. Relative imbalances in the expression of catechol-*O*-methyltransferase and cytochrome P450 in breast cancer tissue and their association with breast carcinoma. *Maturitas* 2012; **72**(2): 139-145.

[4] Kiruthiga PV, Kannan MR, Saraswathi C, Pandian SK, Devi KP. CYP1A1 gene polymorphisms: lack of association with breast cancer susceptibility in the southern region (Madurai) of India. *Asian Pac J Cancer Prev* 2011; **12**(8): 2133-2138.

[5] Szaefer H, Licznerska B, Krajka-Kuzniak V, Bartoszek A, Baer-Dubowska W. Modulation of CYP1A1, CYP1A2 and CYP1B1 expression by cabbage juices and indoles in human breast cell lines. *Nutr Cancer* 2012; **64**(6): 879-888.

[6] Englert NA, Turesky RJ, Han W, Bessette EE, Spivack SD, Caggana M, et al. Genetic and epigenetic regulation of AHR gene expression in MCF-7 breast cancer cells: role of the proximal promoter GC-rich region. *Biochem Pharmacol* 2012; **84**(5): 722-735.

[7] Ptak A, Ludewig G, Rak A, Nadolna W, Bochenek M, Gregoraszczuk EL. Induction of cytochrome P450 1A1 in MCF-7 human breast cancer cells by 4-chlorobiphenyl (PCB3) and the effects of its hydroxylated metabolites on cellular apoptosis. *Environ Int* 2010; **36**(8): 935-941.

[8] Surichan S, Androutsopoulos VP, Sifakis S, Koutala E, Tsatsakis A, Arroo RR, et al. Bioactivation of the citrus flavonoid nobiletin by CYP1 enzymes in MCF7 breast adenocarcinoma cells. *Food Chem Toxicol* 2012; **50**(9): 3320-3328.

[9] Moreno-Galván M, Herrera-González NE, Robles-Pérez V, Velasco-Rodríguez JC, Tapia-Conyer R, Sarti E. Impact of CYP1A1 and COMT genotypes on breast cancer risk in Mexican women: a pilot study. *Int J Biol Markers* 2010; **25**(3): 157-163.

[10] Matsunawa M, Akagi D, Uno S, Endo-Umeda K, Yamada S, Ikeda K, et al. Vitamin D receptor activation enhances benzo[a] pyrene metabolism via CYP1A1 expression in macrophages. *Drug Metab Dispos* 2012; **40**(11): 2059-2066.

[11] Surekha D, Sailaja K, Rao DN, Padma T, Raghunadharao D, Vishnupriya S. Association of CYP1A1*2 polymorphisms with breast cancer risk: a case control study. *Indian J Med Sci* 2009; **63**(1): 13-20.

[12] Wang Q, Li H, Tao P, Wang YP, Yuan P, Yang CX, et al. Soy isoflavones, CYP1A1, CYP1B1, and COMT polymorphisms, and breast cancer: a case-control study in southwestern China. *DNA Cell Biol* 2011; **30**(8): 585-595.

[13] Hafeez S, Ahmed A, Rashid AZ, Kayani MA. Down-regulation of CYP1A1 expression in breast cancer. *Asian Pac J Cancer Prev* 2012; **13**(5): 1757-1760.

[14] Ociepa-Zawal M, Rubis B, Filas V, Breborowicz J, Trzeciak WH. Studies on CYP1A1, CYP1B1 and CYP3A4 gene polymorphisms in breast cancer patients. *Ginekol Pol* 2009; **80**(11): 819-823.

[15] Oleaga C, García M, Solé A, Ciudad CJ, Izquierdo-Pulido M, Noé V. CYP1A1 is overexpressed upon incubation of breast cancer cells with a polyphenolic cocoa extract. *Eur J Nutr* 2012; **51**(4): 465-476.