The clinical significance of gene sequencing analysis of p53 mutation in triple negative breast cancer patients

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Abstract

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Purpose:

The p53 gene mutation rate in breast cancer ranges between 20% and 40%. Missense-type mutations have been particularly associated with worse prognosis in breast cancer. The p53 mutation is more frequently present in 56%–82% of triple-negative breast cancer (TNBC) or basal-like breast cancer cases, and only in 13%–22% of luminal subtypes. Previous some studies had showed p53 expression by immunohistochemistry (IHC) in TNBC had influenced poorer prognosis in TNBC, but others reported that p53 expression by IHC had yielded inferior prognostic information than p53 gene sequencing analysis in breast cancer. Hence, we investigated the clinical value of gene sequencing-based analysis of the p53 mutation in Korean TNBC patients.

Methods:

A total of 476 eligible cases (471 patients), of which 87 were TNBC subtype, who underwent p53 mutation analysis using IHC and polymerase chain reaction-denaturing high performance liquid chromatography (PCR-DHPLC) sequencing between December 2002 and December 2009 were reviewed. The median follow-up

period was 47.5 months (1-100 months). Probabilities for relapse-free survival (RFS), disease-free survival (DFS), breast cancer-specific survival (BCSS), and overall survival (OS) in the TNBC subgroup were estimated using the Kaplan-Meier method, and survival curves for different subgroups were compared by the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model.

Results:

Sixty-eight (14.3%) of the 476 cases had p53 gene mutations detected by PCR-DHPLC sequencing, and 179 cases (37.6%) showed positive IHC staining of p53. Eighty-seven cases (18.3%) were TNBC, of which 22 (25.3%) showed the p53 gene mutation and 51 (58.6%) had positive IHC staining. In the TNBC subgroup, age was the only clinicopathologic factor showing a significant difference between the p53 wild-type and the p53 mutant subtype. Majority of the p53 mutations were found in exon 7 (31.8%), with missense mutation being the most common type (59.1%). Univariate analysis revealed a significant difference in RFS and DFS in p53 missense-positive and p53 missense-negative TNBC patients, as detected by gene sequencing (p=0.035 and 0.041, respectively). BCSS and OS showed trends towards significance (all p = 0.060). In multivariate analysis, the presence of the p53 missense mutation in TNBC patients was a clinically significant prognostic factor for RFS (p=0.040), BCSS and OS (p=0.048, respectively), and showed a trend for DFS (p=0.050).

Conclusion:

The p53 missense mutation have a significant clinical prognostic power in predicting RFS, BCSS, and OS in TNBC patients.

Keywords : missense mutation, p53 gene mutation, PCR-DHPLC sequencing analysis, prognosis, triple negative breast cancer

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I. INTRODUCTION

Human breast carcinomas are a strikingly heterogeneous group of cancers with variable clinical, pathologic, and molecular features[1]. Gene expression studies using DNA microarrays have shown that the breast cancer subtypes are composed of 496 intrinsic genes that differentiate breast cancers into independent groups based on gene expression modes[2-3]. Breast cancer tumors are divided into two main subtypes: estrogen receptor (ER)–negative tumors (basal-like and human epidermal growth factor receptor-2 positive/ER negative [HER2+/ER-] subtype) and two types of ER-positive tumors (luminal A [ER+/HER2-] and luminal B [ER+/HER2+])[4]. Among these molecular subtypes, basal-like tumors typically show low expression of ER, progesterone receptor (PR), and HER2, and high expression of cytokeratin 5, 6, and 17[2]. Additionally, these tumors also frequently show either p53 immunohistochemical (IHC) expression or tumor protein 53 (TP53) gene mutations[4-5].

Triple-negative breast cancers (TNBCs) are characterized by the absence of the ER, PR, and the amplification of the HER2/Neu gene, and comprise 15% to 20% of all breast cancers[6]. Previous studies using DNA microarrays combined with IHC analyses have revealed that 80% to 90% of TNBCs are basal-like, and exhibit a similar clinical behavior[1]. TNBCs display some prominent clinicopathologic and

molecular features: they are more frequent in younger women (<50 years) and African-American women, have a larger mean tumor size and higher grade, show a higher incidence of node positivity at presentation compared to what is expected based on tumor size, are often presented as interval cancers and are significantly more aggressive than other molecular subgroups[7-9].

Previous studies showed that p53 accumulation, as determined by IHC, could be a specific prognostic factor in TNBC[10-11]. However, the results of p53 immunostaining could be heterogeneous according to the type of antibody used, fixation artifacts during the procedure, and so on[12]. Yet another study revealed that a direct cDNA sequence-based analysis of p53 status is superior to IHC in determining prognosis in breast cancer[13].

Along with the lack of therapeutic targets for the treatment of TNBC, we tentatively presumed that an increased frequency of p53 gene mutations in TNBC than in the luminal subtypes might play a crucial role in the poor prognosis and aggressive clinical outcome of this cancer. However, to our knowledge, the p53 status in TNBC has not been investigated using gene sequence analysis, in contrast to IHC analysis, which is the most common method for evaluating the correlation between p53 status and cancer prognosis. Hence, in this study, we evaluated the prognostic significance of the p53 gene mutation in TNBCs using DNA sequence-based analysis.

II. MATERIALS AND METHODS

1. Study population

A. Patients

In this study, a dataset of 1,340 Korean women who were diagnosed and treated for breast cancer between December 2002 and December 2009 at the Breast Cancer Center, Gangnam Severance Hospital was retrospectively reviewed for their eligibility for this study. This study was approved by the Gangnam Severance Hospital institutional review board.

B. Eligibility

Patients who were not diagnosed with TNBC, who did not undergo p53 gene sequencing due to any reason, who were treated with neoadjuvant chemotherapy, and who were diagnosed with synchronous bilateral breast cancer or ductal carcinoma in situ (DCIS) were excluded from this study. All eligible patients were followed up for recurrence and survival.

C. Relapse-free survival, disease-free survival, breast cancer-specific survival, and overall survival

Relapse-free survival (RFS) was defined as the time between initial diagnosis and locoregional recurrence (LRR), distant metastasis, and death from any cause. Disease-free survival (DFS) was defined as the time between initial diagnosis and LRR, distant metastasis, secondary same and/or other cancer, and death from any cause. Breast cancer-specific survival (BCSS) was defined as the time between initial diagnosis and death from any cause related to breast cancer. Overall survival (OS) was defined as the time between initial diagnosis and death from any cause, regardless of recurrence events. LRR or distant recurrence was confirmed by reviewing imaging or pathologic reports after biopsy.

D. Clinical follow-up

Patient follow-up was implemented every 6 months in the first 5 years after surgery, and every 1 year thereafter. In the first 5 years, results of posteroanterior chest X-ray, mammography, and breast ultrasonography were basically evaluated every 6 months. Chest and abdominal-pelvic computed tomography (CT), whole-body bone scan (WBBS), and positron emission tomography (PET) CT scan were additionally examined every 1 year. After the first 5 years, the above examinations were repeatedly performed every 1 year. Physical examination and basic blood tests such as complete blood count were performed every 1 year, unless any abnormal results were detected.

2. Molecular subgrouping based on IHC analysis

The ER-negative group was divided into two subgroups as the HER2+/ER- type and the TNBC type (ER-/HER2-). The ER-positive group was divided into the luminal A type (ER+/HER2-) and the luminal B type (ER+/HER2+). Because there were no routine evaluations for cytokeratins in our institution, the basal-like type was substituted by the TNBC type. If the HER2 status was equivocal based on IHC results, we proceeded to the fluorescence in situ hybridization (FISH) test for evaluation of HER2 gene amplification.

3. p53 gene mutation analysis

Mutational analysis of exons 5–9 of the p53 gene was carried out using polymerase chain reaction–denaturing high performance liquid chromatography (PCR-DHPLC) and direct sequencing.

A. Extraction of genomic DNA

About 1 mg samples from either biopsies or surgical specimens, freshly frozen or paraffin-embedded, were cut into small pieces. Extraction of DNA was performed

using the Easy-DNA^{TM} kit (Invitrogen, Carlsbad, CA, USA), and 100 ng/ul of DNA was used for each PCR reaction.

B. PCR amplification

Each PCR was performed in a 20 μl reaction mixture containg 100 ng of DNA, 20 μmol of forward and reverse primers, 2 μl of Taq buffer(10×), 2.5 mM of deoxyribonucleotide triphosphates (dNTPs), 2.5 mM of $MgCl_2$, and 0.7 U of Taq DNA polymerase. PCR conditions were 95°C for 5 min; 50 cycles of 94°C for 10 sec, 62°C for 10 sec, 72°C for 15 sec; and 72°C for 5 min in a DNA thermal cycler (Perkin-Elmer, GeneAmp PCR System 2400, USA). The PCR products were kept under 4°C until further analysis. Each PCR product was first screened for mutations by denaturing high-performance liquid chromatography (DHPLC)(WAVE; transgenomic, Omaha, Nebraska, USA) and followed by sequence analysis if heteroduplex formation is detected. The protocol for DHPLC is provided in section C. After mixing 20 μl of each exon PCR product with an equal amount of the corresponding wild type PCR product, the mixture was incubated at 95°C for 5 minutes, and then kept at room temperature.

Table 1 Primer sequence used to amplify p53 gene

		Sequence of primer	Length(bp)
exon 5	Forward	5′-ATCTgTTCACTTgTgCCCTg	274
	Reverse	5′-AACCAgCCCTgTCgTCTCTC	
exon 6	Forward	5′-AgggTCCCCAggCCTCTgAT	197
	Reverse	5′-CACCCTTAACCCCTCCTCCC	
exon 7	Forward	5′-CCAAggCgCACTggCCTCATC	205
	Reverse	5′-CAgAggCTggggCACAgCAgg	
exon 8	Forward	5′-TTCCTTACTgCCTCTTgCTT	194
	Reverse	5′-TgTCCTgCTTgCTTACCTCg	
exon 9	Forward	5′-CgCCgTgCAgTTATgCCTCAgATTC	279
	Reverse	5′-CCCCCgCCCggCCCCAATTgCAggTAAAAC	

C. Denaturing High Performance Liquid Chromatography (DHPLC)

(A) Procedures

ⓐ Separation of heteroduplex and homoduplex strands

Triethylammonium acetate (TEAA), which has an amphiphilic character, was first absorbed into the surface of the DNASep cartridge (Transgenomic, USA) through an association with the negatively charged phosphate backbone of DNA. Elution of the DNA products was carried out using acetonitrile (ACN), which has a higher positive charge than TEAA. This way, the heteroduplexes and short strands are more rapidly separated than the homoduplexes and long strands.

ⓑ Mobile phase

A gradient solution of buffer A (0.1M TEAA solution, pH 7.0) and buffer B (0.1M TEAA and 25% ACN, pH 7.0) was used. The cleansing solution was used with buffer C (8% ACN [syringe washing solution]) and with buffer D (75% ACN [DNASep Cartridge UltraClean and Storage Solution]).

ⓒ Stationary phase

The DNASep Cartridge (Transgenomic, USA) column is available as an

alkylated nonporous poly(styrene-divinylbenzene) form. The column was washed with buffer D at 0.9 ml/min at 60°C for 30 minutes, and stabilized using the mixture of Buffers A and B (mixed in the same proportion) at 0.9 ml/min for 60 minutes.

ⓓ Detection of separated DNA

To check the purity of the PCR products, 0.5 μl of the specimen, which was not denaturalized, was injected into the column at 0.9 ml/min at 50 °C, after which the temperature of the column was elevated to 63 °C. The eluted DNA was detected using an ultra-violet light detector at a wavelength of 260 nm.

(B) Analysis of chromatographic products

Because heteroduplexes are associated with the column through weak interactions, they can be more rapidly eluted out of the column than the homoduplexes, and thus, appear as separate forms in the chromatogram.

(C) The DHPLC device was operated according to the manufacturer's instructions. After the denaturation of the PCR products at 95°C for 5 minutes, annealing was performed at 55°C for about 40 minutes. The separation of these products, in the form of a chromatogram, was monitored on a computer screen. In general, heterogeneous molecules had one more peak in addition to that observed for the homozygous molecules, whereas the homozygous molecules had only one peak.

D. Sequence analysis

Sequencing was performed using commercial reagents and an automated sequencer (ABI Prism BigDye Terminator v 3.1 cycle sequencing kit and ABI 310 Genetic Analyzer; Applied Biosystems,Foster city, USA). Both forward and reverse strands were sequenced to confirm the nucleotide alterations.

4. Statistical analysis

Statistical analysis was performed using the SPSS software (version 18, Chicago, IL, USA). To compare the frequency distribution of the clinicopathologic

characteristics, the chi-square test and the student's t-test were used. Univariate survival analysis (RFS, DFS, BCSS and OS) of the p53 mutation status and of each of the prognostic factors was performed using the log-rank test with the Kaplan-Meier survival curves. Multivariate analysis was carried out using the Cox proportional hazards model. The prognostic significance of each factor was summarized by relative risk (RR), as estimated by the hazard ratio in the Cox proportional hazards model.

III. RESULTS

1. Patient and tumor characteristics

Between December 2002 and December 2009, a total of 1,340 cases were reviewed. The median follow-up period was 47.5 months (1-100months). The following cases were excluded from the study: 766 cases with no p53 gene sequencing data available, 21 cases of DCIS, 14 cases of DCIS with microinvasion (MI), 43 cases of preoperative chemotherapy, 19 cases of synchronous bilateral breast cancer, and 1 case which could not analyzed for p53 mutation subtype. Thus, a total of 476 cases (471 patients) were eligible for the inclusion criteria of this study. Among these patients, 251 cases (52.7%) were luminal A subtype, 65 cases (13.7%) were luminal B subtype, 72 cases (15.1%) were HER2+/ER- subtype, and 87 cases (18.3%) were TNBC subtype. One patient was excluded because of unknown hormone and HER2 status. The clinicopathologic characteristics of the TNBC subtype are given in Table 2. Age was the only clinicopathologic factor that showed a statistically significant difference between the p53 wild-type subtype and the p53 mutant subtype in TNBC. Tumor subtype, tumor size, nuclear and histologic grade, extensive intraductal component (EIC), lymphovascular invasion (LVI) of the primary tumor, multifocality, metastatic axillary nodal status, perinodal extension, Ki67, and p53 protein status analyzed by IHC were not significantly different between the two subgroups. Further, there was no significant difference in the type of adjuvant treatments such as chemotherapy and radiotherapy between the two subgroups.

Table 2. Clinicopathologic characteristics in TNBC patients

	Number	p53 wild-type	p53 Mutation	Mutated(%)	*p*
F/U months, median *	87	42 (1-98)	56 (3-78)		0.840
Age (years)					0.002
<50	47	29	18	38.3	
≥50	40	36	4	10.0	
Tumor subtype†					0.337
Ductal	72	52	20	27.8	
Other	15	13	2	13.3	
Tumor size†					1.000
T1	29	21	8	27.6	
T2	46	33	13	28.3	
T3	4	3	1	25.0	
T4	1	1	0	0	
Nuclear grade†					0.824
1	3	3	0	0	
2	29	21	8	27.6	
3	49	35	14	28.6	
Histologic grade†					0.427
1	5	5	0	0	
2	26	18	8	30.8	
3	50	36	14	28.0	
LVI					1.000
Absence	69	51	18	26.1	
Presence	10	8	2	20.0	
Multifocality					1.000
Absence	83	62	21	25.3	
Presence	4	3	1	25.0	
Nodal status†					0.703
N0	52	38	14	26.9	
N1	15	10	5	33.3	
N2	8	5	3	37.5	
N3	3	3	0	0	
Perinodal extension†					0.541
Absence	45	29	16	35.6	
Presence	2	2	0	0	
Ki67					0.570
Low	41	31	10	24.4	
High	40	28	12	30.0	
p53 protein by IHC					0.449
Negative	32	25	7	21.9	
Positive	51	36	15	29.4	
Chemotherapy†					0.411
Done	69	51	18	26.1	
Not done	5	3	2	40.0	
Radiiotherapy					0.400
Done	54	45	12	22.2	
Not done	33	23	10	30.0	

TNBC, triple negative breast cancer; LVI, lymphovascular invasion; IHC, immunohistochemistry

* Student's t-test, † Fisher's exact test

2. Analysis of the p53 mutation by gene sequencing and of the p53 protein status by IHC

In a total of 476 cases, the p53 mutation was found in 68 cases (14.3% using gene sequencing), and a positive p53 protein status was found in 179 cases (37.6%) using IHC. Of the 179 cases which showed a positive p53 status by IHC, p53 gene mutations were detected in 45 cases (25.1%) using the DHPLC method, whereas of the 261 cases which showed a negative p53 status by IHC, p53 gene mutations were found in 23 cases (8.8%) using sequencing analysis. Thirty-six cases were not available for IHC analysis (Table 3). Majority of the p53 mutations were located in exon 7 (20 cases, 29.4%; Table 4). Missense mutations of the p53 gene were most common (42 cases, 61.8%; Table 5).

The TNBC subtype along with the HER2+/ER- subtype exhibited more frequent p53 gene mutations than the luminal A and B subtypes, and this difference was statistically significant (p=0.000; Table 6). In the TNBC subtype, exon 7 was the most common site of p53 mutations (7 cases, 31.8%; Table 4), and the most common mutations were the missense type (13 cases, 59.1%; Table 5).

Table 3. Number of cases negative and positive for p53 mutations according to IHC- and gene sequencing-based analysis in total 476 breast cancer cases and TNBC 87 cases. Thirty six cases in total and 4 cases in TNBC were excluded due to unavailable IHC data

Total 476 cases			
	p53 gene mutation		
p53 status by IHC	Wild-type (%)	Mutation (%)	Total
Negative (%)	238 (91.2%)	23 (8.8%)	261
Positive (%)	134 (74.9%)	45 (25.1%)	179
Total	372	68	440
TNBC 87 cases			
	p53 gene mutation		
p53 status by IHC	Wild-type (%)	Mutation (%)	Total
Negative (%)	25 (78.1%)	7 (21.9%)	32
Positive (%)	36 (70.6%)	15 (29.4%)	51
Total	61	22	83

IHC, immunohistochemistry; DHPLC, denaturing high performance liquid chromatography; TNBC, triple negative breast cancer

Table 4. Locations of p53 mutations in total 476 cases and TNBC 87 cases

	Exon 5	Exon 6	Exon 7	Exon 8	Exon 9
Total 476 cases	19 (27.9%)	13 (19.1%)	20 (29.4%)	10 (14.7%)	5 (7.4%)
TNBC 87 cases	5 (22.7%)	5 (22.75)	7 (31.8%)	4 (18.2%)	1 (4.5%)

TNBC, triple negative breast cancer

Table 5. Types of p53 mutations in total 476 cases and TNBC 87 cases

	Missense	Nonsense	Splicing	Frame deletion	Frame shift	Insertion	Silence
Total 476 cases	42 (61.8%)	7 (10.3%)	6 (8.8%)	0	8 (11.8%)	3 (4.4%)	1 (1.5%)
TNBC 87 cases	13 (59.1%)	3 (13.6%)	1 (4.5%)	0	4 (18.2%)	1 (4.5%)	0

TNBC, triple negative breast cancer

Table 6. Comparison of p53 status by IHC- and gene sequencing analysis between molecular subgroups in total 476 cases

	Luminal A (n=251)	Luminal B (n=65)	HER2+/ER- (n=72)	TNBC (n=87)
p53 positive by IHC	59 (23.5%)	27 (41.5%)	42 (58.3%)	51 (58.6%)
p53 mutation	13 (5.2%)	11 (16.9%)	22 (30.6%)	22 (25.3%)

IHC, immunohistochemistry; DHPLC, denaturing high performance liquid chromatography; TNBC, triple negative breast cancer

3. Recurrences and deaths

Among the total 476 cases with the median follow up period of 47.5 months, there were 11 cases (2.3%) of LRRs, 24 cases (5.0%) of systemic recurrences (SRs), and 4 cases (0.8%) of combined LRRs and SRs. Breast cancer-related deaths constituted 18 cases (3.8%), and non-breast cancer-related deaths constituted 3 cases (0.6%).

In the TNBC subtype (87 cases), there were 2 cases (2.3%) of LRRs, 8 cases (9.2%) of SRs, and 2 cases (2.3%) of combined LRRs and SRs. Breast cancer-related deaths constituted 8 cases (9.2%), and no non-breast cancer-related deaths were reported.

4. Univariate and multivariate analysis of RFS, DFS, BCSS, and OS in the TNBC subtype

In the univariate comparison, T stage, N stage, the presence of perinodal extension, and the presence of p53 missense mutation were statistically significant (Table 7) for RFS, DFS, BCSS, and OS. Histologic grade was only statistically significant for BCSS and OS (p=0.024 for both). The p53 missense mutation was not statistically significant for BCSS and OS; however, showed a trend towards significance (p=0.060 for both; Figure 1).

In the multivariate analysis, tumor size, number of metastatic axillary lymph nodes, and the presence of p53 missense mutation were statistically significant for RFS, DFS, BCSS and OS, except tumor size, which was not a significant predictor for

DFS (p=0.136), BCSS, and OS (p=0.062 for both). The relative risk of the presence of p53 missense mutation was 4.053 for RFS (p=0.040), 3.086 for DFS (p=0.050), and 5.297 for BCSS and OS (p=0.048 for both; Table 8).

Table 7. Univariate analysis by log-rank test of clinicopathologic variables in TNBC patients

	RFS	DFS	BCSS, OS
	p	*p*	*p*
Age (<50 vs ≥50)	0.788	0.750	0.192
Tumor subtype (IDC vs Other)	0.563	0.410	0.227
T stage	0.000	0.000	0.000
Nuclear grade	0.330	0.507	0.073
Histologic grade	0.092	0.208	0.024
LVI	0.779	0.948	0.608
Multifocality	0.602	0.589	0.653
N stage	0.000	0.004	0.000
Perinodal extension	0.000	0.000	0.000
Ki67 (Low vs High)	0.124	0.061	0.171
Chemotherapy	0.572	0.720	0.438
Radiotherapy	0.982	0.798	0.953
p53 positivity by IHC	0.266	0.648	0.497
p53 mutation	0.348	0.060	0.438
p53 missense mutation vs other	0.035	0.041	0.060

TNBC, triple negative breast cancer; LVI, lymphovascular invasion; IHC, immunohistochemistry; RFS, relapse-free survival; DFS, disease-free survival; BCSS, breast cancer-specific survival; OS, overall survival

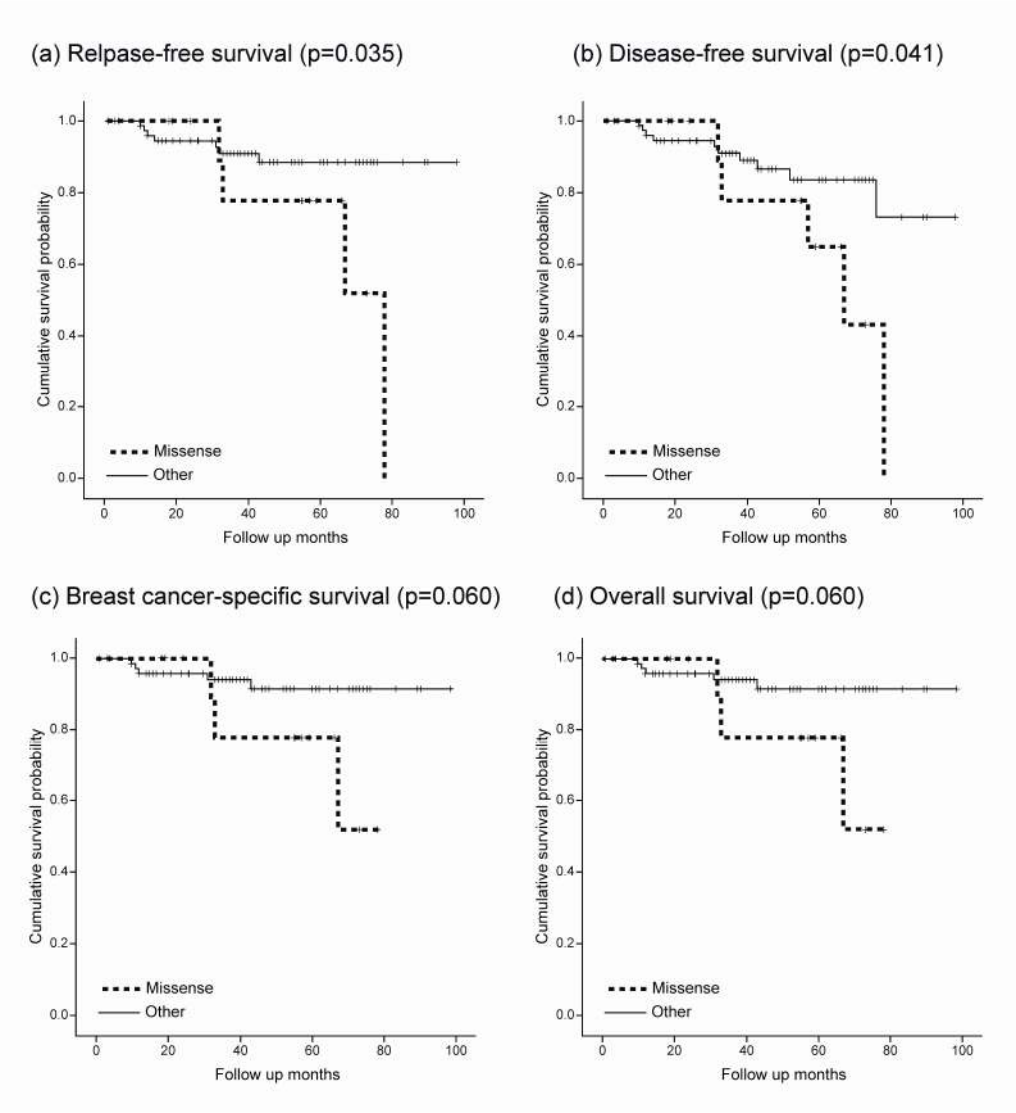

Figure 1. Kaplan-Meier RFS (a), DFS (b), BCSS (c), and OS (c) curves stratified by presence of p53 missense mutation in TNBC patients

TNBC, triple negative breast cancer; RFS, relapse-free survival; DFS, disease-free survival; BCSS, breast cancer-specific survival; OS, overall survial

Table 8. Multivariate analysis by Cox proportional hazards model in TNBC patients

	RFS		DFS		BCSS, OS	
	RR (95% CI)	*p*	RR (95% CI)	*p*	RR (95% CI)	*P*
Tumor size	1.751 (1.029-2.980)	0.039	1.444 (0.891-2.338)	0.136	1.802 (0.971-3.345)	0.062
Number of metastatic axillary lymph node	1.223 (1.072-1.396)	0.003	1.172 (1.036-1.326)	0.011	0.233 (1.059-1.436)	0.007
p53 mutation : missense vs other	4.053 (1.065-15.428)	0.040	3.086 (1.000-9.525)	0.050	5.297 (1.016-27.601)	0.048

TNBC, triple negative breast cancer; RFS, relapse-free survival; DFS, disease-free survival; BCSS, breast cancer-specific survival; OS, overall survial

IV. DISCUSSION

This study showed that the p53 mutation, in particular, the missense type, was a significant prognostic factor for RFS, BCSS, and OS in Korean patients with the TNBC subtype. For DFS, the p53 missense mutation in the TNBC subtype could not be established as a determinant factor for poor prognosis, but showed a trend towards significance.

The p53 gene is located on the short arm of chromosome 17 (17p13.1), is composed of 11 exons (about 20 kb), and is known as the gatekeeper gene.[14] Previous studies reported that the loss of normal p53 gene function is correlated with poor prognosis, and the p53 gene mutation rate in breast cancer is between 20% and 40%.[15-16] Although most of the previous studies on p53 gene mutation analysis and p53 protein manifestation used IHC or single-strand conformation polymorphism (SSCP), these techniques have some limitations. The accuracy of detection using IHC was reported to be only between 80% and 85%, and the SSCP method has some shortcomings, such as variable sensitivity observed by investigators (60%–90%), a false negative rate of 5%–8%, high costs, and technical difficulties that could be time-consuming.[17] In contrast, the DHPLC method for analysis of the p53 gene mutation showed some advantages, such as high sensitivity and specificity between 96% and 100%, relatively low costs, and the use of automated devices that can save time[17-21]. Hence, we investigated the clinical value of DHPLC-based sequencing analysis of the p53 gene mutation in breast cancer patients. Among a total of 476 breast cancer cases, 68 cases (14.3%) had p53 gene mutations. Our study also revealed that exon 7 (20 cases, 29.4%) of the p53 gene was the most common site for the occurrence of mutations, and the missense type (42 cases, 61.8%) was the most common p53 mutation type. Similarly, in the TNBC subtype, exon 7 was the most common site (7 cases, 31.8%), and the missense mutation (13 cases, 59.1%) was the most common type of p53 gene mutation. Our findings differ from those of previous studies. Carey et al.[3] in the Carolina Breast Cancer Study showed that 84 cases (25%) of the total 330 cases

exhibited the p53 mutation, as detected by SSCP analysis. Based on the molecular subtypes, they reported that rates of p53 mutations were 43% in the basal-like subtype, 44% in the HER2+/ER- subtype, 23% in the luminal A subtype, and 15% in the luminal B subtype. Sjögren et al.[13] reported that 22% of the 316 primary breast tumors under study harbored p53 mutation, as analyzed by the cDNA-based sequencing method. Visscher et al.[12] reported that 50% of the 18 cases, which were selected for analysis from 82 infiltrating ductal carcinomas of the breast, carried the p53 mutation, as diagnosed by using SSCP. Olivier et al.[21] reported that in a total of 308 samples analyzed for p53 mutations based on a sequencing approach using constant denaturing gel electrophoresis, denaturing gradient gel electrophoresis, temporal gradient electrophoresis, SSCP, and DHPLC sequencing, a single mutation within exon 5 to 8 (17%, 308 of 1,794 cases) as well as codon 248 (11%, 26 of 235 missense mutations), codon 273 (8.5%, 20 of 235 missense mutations), and codon 175 (8.1%, 19 of 235 missense mutations) were detected as commonly occurring p53 mutations. Bull et al.[22] revealed that p53 mutations detected by SSCP occurred in 24.5% of the axillary node-negative breast carcinomas in a total of 543 patients. In this study, the frequency of p53 mutations (68 of 476, 14.3%) was lower than that in other studies, and codons with the most frequently occurring p53 mutations were 175 (6.6%), 238 (4.9%), 637 (4.9%), and 722 (4.9%). These discrepancies in the incidence and location of p53 mutations observed between previous studies and our study may be due to ethnic differences in the study patients and/or due to heterogeneous analytical methods and samples, although this is not clear. Further investigations are needed to address these discrepancies.

TNBCs are characterized by an aggressive clinical history and poor clinical outcome.[1,9,23-25] In a retrospective analysis of 49 patients with basal-like breast cancer (BLBC), DFS and OS were inferior to age-matched, stage-matched, and grade-matched controls expressing non-basal-like cytokeratin.[26] Two studies have shown a trend of a poorer outcome in patients with TNBC as compared to patients with the ErbB2-positive type.[27-28] Furthermore, TNBC patients show trends of an earlier age of

onset[29] and a high rate of local relapse[30] as well as a higher incidence of visceral metastases than bone metastases,[25,29-30] and cerebral metastasis.[31] Irvin et al.[24] evaluated the histologic features of TNBC, which were generally a higher histologic grade, elevated mitotic count, scant stromal content, central necrosis, pushing margins of invasion, a stromal lymphocytic response, and multiple apoptotic cells that were histologically largely ductal; however, several unusual histological features were also overrepresented, including metaplastic, atypical or typical medullary, or adenoid cystic carcinomas. Furthermore, the authors commented that high p53 expression in IHC or presence of TP53 gene mutations were common in BLBC; in one study 82% of BLBC cases had p53 mutations compared to only 13% in the luminal A subtype ($p<0.001$).[23-24] Our study revealed that the rate of p53 mutation in TNBC (25.3%) was higher than in luminal A (5.2%) and B (16.9%) subtypes ($p = 0.000$). Also interestingly, especially younger age patients (< 50 years) had p53 mutations nearly four times more often than older age patients (≥ 50 years) ($p = 0.002$). But this result should be interpreted cautiously because of insufficient number of patients to conclude that.

The fundamental reasons for a poor clinical outcome of TNBC patients may be related to its basically aggressive histologic features and the lack of specific targets for treatment, such as ER and HER2. Moreover, as demonstrated in the present study, a higher rate of p53 mutation in TNBC than in the luminal type, especially of the missense type of mutations, may have a significant influence on prognosis. Previous studies on correlation between p53 status and TNBC showed that a p53-expressing tumor had worse survival; however, the p53 status in this study was assessed using IHC, and a restricted number of TNBC subgroups were analyzed, such as patients treated with adjuvant anthracycline-based chemotherapy or patients with node negative breast cancer.[10-11,32]

Sjögren et al. [13] reported that use of a cDNA-based sequencing method to determine the status of the p53 gene in primary breast cancers yielded better prognostic information than IHC performed with the Pab 1801 monoclonal antibody. They

reported that the false-negative rate (positive by sequencing and negative by IHC) was 33% (23 of 69 cases), and the false-positive rate (negative by sequencing and positive by IHC) was 30% (19 of 64 cases). Possible explanations for false-negative results were the presence of premature stop codons and gross deletion of the p53 gene, which could lead to a cessation of protein synthesis and make it impossible to detect p53 status by IHC. Another possible mechanism suggested was that the genetic alterations caused changes in or the disappearance of the epitope recognized by Pab 1801. It was further suggested in this report that that missense mutations, as well as deletions, insertions, and premature stop codons, might produce conformational changes in the p53 polypeptide that interfere with recognition of the specific epitope by Pab 1801. Additionally, truncation of the carboxyl terminus of p53 might reduce the stability of the mutated protein.[33] To account for the false-positive results, they suggested that, in some cases, the accumulation of p53 in tumor cells may indicate the existence of a regulatory defect rather than mutations in the protein-coding sequence of the gene, and in some cases, weak immunostaining due to mild reactivity of the wild-type p53 cDNA sequences could also be a cause of IHC positivity. Our results indicated similar false-negative (33.8% in total 476 cases, 31.8% in TNBC 87 cases) and false-positive rate (36.0% in total, 34.8% in TNBC) with previous described study. However, in contrast to previous manuscripts,[10-11] all survivals showed no statistically significant differences by p53 expression tested with IHC (RFS, DFS, BCSS and OS; $p > 0.05$). This may be explained by clinicopathologic heterogeneity of each study population, discordance of testing method of p53 expression in each of the institutions, and a relative small number of our study population (TNBC 87 cases) with relatively short term follow up period to occur consecutive events.

Olivier et al.[21] showed that the prognostic value of TP53 mutation in 1,794 patients with breast cancer was independent of tumor size, node status, and hormone receptor content, and TP53 mutation combined with the absence of the progesterone receptor (PR) was associated with the worst prognosis. In addition, they revealed that missense mutations, especially in the DNA-binding motifs, (although non-missense mutations

in DNA-binding motifs had similar prognostic value) and specific missense mutants (codons 179 and R248W) have worse prognosis. The present study also showed that the p53 missense mutation was independent of tumor size and node status in predicting RFS, BCSS, and OS in TNBC. However, because of the small number of cases studied, we could not verify whether a specific codon in TNBC can have different prognostic values.

This study was performed by evaluating all cases diagnosed with primary invasive breast carcinoma, and only PCR-DHPLC-sequencing were used as a homogeneous analytical method for evaluating p53 gene mutations, the results of which were compared with IHC. Our study indicates that the p53 missense mutation in the TNBC subtype offers a significant prognostic power for RFS, BCSS, and OS in the same way as tumor size, number of axillary metastatic lymph nodes which were most powerful prognostic factors, and even higher relative risks as roughly three-to-four times than universal prognostic factors, regardless of slightly marginal statistical significances. For DFS, the p53 missense mutation in TNBC has a trend towards being a prognostic factor, but the result was not statistically significant (p=0.050). We anticipate that a longer follow-up period will help the p53 missense mutation in TNBC to gain sufficient prognostic power to predict DFS.

Bull et al.[22] reported a significant and persistent risk of recurrence and poor overall survival associated with a p53 mutation in combination with neu/erbB-2 amplification in node-negative breast cancer. Ozcelik et al.[34] showed that missense mutations in combination with ERBB2 amplification dramatically affected the disease-specific survival (DSS) and DFS in node-negative breast cancer. We need to validate this finding by comparing the HER2+/ER- subgroup with the TNBC subgroup.

V. CONCLUSION

To investigate the clinical significance of the p53 mutation in TNBC, we reviewed 476 cases (471 patients who were analyzed for p53 mutations using PCR-DHPLC-sequencing between December 2002 and December 2009). Based on our findings, we propose that a high rate of p53 mutations in TNBC would have a specific clinical prognostic value. Analysis of the p53 mutation using PCR-DHPLC coupled with gene sequencing was not too meaningful for prognosis; however, when stratified by subtypes, the p53 missense mutation proved to have a significant clinical prognostic power in predicting RFS, BCSS, and OS, and showed a trend to be of prognostic value for DFS. To our knowledge, this study is the first to report the clinical value of p53 missense mutations in TNBC. Future attempts would be directed at applying the prognostic value of p53 missense mutations to treat TNBC, which is highly challenging to manage due to the absence of specific and powerful therapeutic targets such as ER and HER2.

REFERENCES

1. Rakha EA, El-Sayed ME, Green AR, Lee AHS, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. Cancer 2007;109:25-32.

2. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000;406:747-52.

3. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006;295:2492-502.

4. Sorile T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnson H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869-74.

5. Calza S, Hall P, Auer G, Bjöhle, Klaar S, Kronenwett U, et al. Intrinsic molecular signature of breast cancer in a population-based cohort of 412 patients. Breast Cancer Res 2006;8:R34.

6. Silver DP, Richardson AL, Ekund AC, Wang ZC, Szallasi Z, Li Q, et al. Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. J Clin Oncol 2010;28:1145-53.

7. Dolle JM, Daling JR, White E, Brinton LA, Doody DR, Porter PL, et al. Risk factors for triple-negative breast cancer in women under the age of 45 years. Cancer Epidemiol Biomarkers Prev 2009;18:1157-65.

8. Perez EA, Moreno-Aspitia A, Thompson EA, Andorfer CA. Adjuvant therapy of triple negative breast cancer. Breast Cancer Res Treat 2010;120:285-91.

9. Reis-Filho JS, Tutt AJN. Triple negative tumours. Histopathology 2008;52:108-18.
10. Chae BJ, Bae JS, Lee A, Park WC, Seo YJ, Song BJ, et al. p53 as a specific prognostic factor in triple-negative breast cancer. Jpn J Clin Oncol 2009;39:217-24.

11. Biganzoli E, Coradini D, Ambrogi F, Garibaldi JM, Lisboa P, Soria D, et al. p53 status identifies two subgroups of triple-negative breast cancers with distinct biological features. Jpn J Clin Oncol 2011;41:172-9.

12. Visscher DW, Sarkar FH, Shimoyama RK, Crissman JD. Correlation between p53 immunostaining patterns and gene sequence mutations in breast carcinoma. Diagn Mol Pathol 1996;5:187-93.

13. Sjögren S, Inganäs M, Norberg T, Lindgren A, Nordgren H, Holmberg L, et al. The p53 gene in breast cancer: prognostic value of complementary DNA sequencing versus immunohistochemistry. J Natl Cancer Inst 1996;88:173-82.

14. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88:323-1.

15. Sturm I, Papadopoulos S, Hillebrand T, Benter T, Luck HJ, Wolff G, et al. Impaired BAX protein expression in breast cancer: mutational analysis of the BAX and the p53 gene. Int J Cancer 2000;87:517-21.

16. Barnes DM, Camplejohn RS. P53, apoptosis, and breast cancer. J Mamm Gl Biol Neoplasia 1996;1:163-75.

17. Gross E, Arnold N, Goette J, Schwarz-Boeger U, Kiechle M. A comparison of BRCA1 mutation analysis by direct sequencing, SSCP and DHPLC. Hum Genet 1999;105:72-8.

18. Keller G, Hartmann A, Mueller J, Höfler H. Denaturing High Pressure Liquid Chromatography (DHPLC) for the analysis of somatic p53 mutations. Laboratory Investigation 2001;81:1735-7.

19. Xiao W, Oefner PJ. Denaturing high-performance liquid chromatography: A reviw. Hum Mutat 2001;17:439-74.

20. Frueh FW, Noyer-Weidner M. The use of denaturing high-performance liquid chromatography (DHPLC) for the analysis of genetic variations: Impact for diagnostic and pharmacogenetics. Clin Chem Med 2003;41:452-61.

21. Olivier M, Langerød A, Carrieri P, Bergh J, Klaar S, Eyfjord J, et al. The clinical value of somatic TP53 gene mutations in 1,794 patients with breast cancer. Clin Cancer Res 2006;12:1157-67.

22. Bull SB, Ozcelik H, Pinnaduwage D, Blacksetin ME, Sutherland AJ, Pritchard KI, et al. The combination of p53 mutation and neu/erbB-2 amplification in associated with poor survival in node-negative breast cancer. J Clin Oncol 2004;22:86-96.

23. Cleator S, Heller W, Coombes RC. Triple-negative breast cancer:therapeutic options. Lancet Oncol 2007;8:235-44.

24. Irvin Jr. WJ, Carey LA. What is triple-negative breast cancer? Eur J Cancer 2008;44:2799-805.

25. Dent R, Hanna WM, Trudeau M, Rawlinson E, Sun P, Narod PS. Pattern of

metastatic spread in triple-negative breast cancer. Breast Cancer Res Treat 2009;115:423-8.

26. Banerjee S, Reis-Filho JS, Ashley S, Steele D, Ashworth A, Lakhani SR, et al. Basal-like breast carcinomas: clinical outcome and response to chemotherapy. J Clin Pathol 2006;59:729-35.

27. Solie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003;100:8418-23.

28. Abd El-Rehim DM, Ball G, Pinder SE, Rhaka E, Paish C, Robertson JF, et al. High-throughput protein expression analysis using tissue microarray technology of a large well-characterized series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. Int J Cancer 2005;116:340-50.

29. Osborne CR, Kannan L, Ashfaq R, Ariyibi J, Frawley WH, Tripathy D. Clinical and pathological characterization of basal-like breast cancer. Breast Cancer Res Treat 2005;94(Suppl 1):S118 Abstract 2098.

30. Rodriguez-Pinilla SM, Sarrio D, Honrado E, Hardisson D, Calero F, Benitez J, et al. Prognostic significance of basal-like phenotype and fascin expression in node-negative invasive breast carcinoma. Clin Cancer Res 2006;12:1533-9.

31. Tsuda H, Takarabe T, Akashi-Tanaka S, Fukutomi T, Nanasawa T, Watanabe T. Large, central acellular zones indicating myoepithelial tumor differentiation in high-grade invasive ductal carcinomas as markers of predisposition to lung and brain metastasis. Am J Surg Pathol 2000;24:197-202.

32. Jung SY, Jeong J, Shin SH, Kwon YM, Kim EA, Ko KL, et al. Accumulation of p53 determined by immunohistochemistry as a prognostic marker in node negative breast cancer; analysis according to St Gallen Consensus and intrinsic subtypes. J Surg Oncol 2011;103:207-11.

33. Ohuc M, Tomita N, Monden T, Fujita M, Fukunaga M, Takami K, et al. A frequent alteration of p53 gene in carcinoma in adenoma of colon. Cancer Res 1994;54:4798-804.

34. Ozcelik H, Pinnaduwage D, Bull SB, Andrulis IL. Type of TP53 mutation and ERBB2 amplification affects survival in node-negative breast cancer. Breast Cancer Res Treat 2007;105:255-65.

국문요약

삼중음성 유방암군에서 p53 돌연변이의 유전자 염기서열 분석의 임상적 의의

목적 :

인간에게 발생하는 다양한 암종에서 관찰되는 p53 종양억제유전자의 돌연변이는 유방암에서 약 20%에서 40%까지 보고되고 있다. 이전 연구에 의하면 특히 과오돌연변이(missense mutation)가 유방암에 있어서 더욱 나쁜 예후와 연관이 있다고 알려져 있다. 삼중음성 유방암 (triple negative breast cancer, TNBC) 혹은 기저세포양 유방암 (basal-like breast cancer)에 대한 일부 연구에서 p53 돌연변이는 56%에서 82%까지 더욱 빈번한 것으로 보고되어 내강형 유방암 (luminal type breast cancer)에서의 13%에서 22%보다도 많이 발견됨이 보고 되었다. 이전 일부 연구에서 면역조직화학법을 통한 p53 발현이 삼중음성 유방암에서 불량한 예후에 영향을 미친다는 보고를 하였으나, 또 다른 연구에서 면역조직화학법에 의한 p53 발현이 p53 유전자 염기서열 분석을 통한 분석에 비해서 유방암의 예후예측력이 다소 낮은 것으로 보고되기도 하였다. 이에 본 연구는 삼중음성유방암 환자군을 대상으로 p53 돌연변이를 유전자 염기서열 분석방법으로 분석하여 그 임상적인 예후적 의의를 살펴보았다.

방법 :

본 연구는 2002년 12월부터 2009년 12월까지 강남세브란스병원에서 유방암을 진단받고 치료받았던 환자들 중 p53 돌연변이에 대한 유전자 염기서열 분석방법(polymerase chain reaction (PCR)-denaturing high performance chromatograpy (DHPLC)-sequencing)과 면역조직화학법으로 검사가 이루어진

476예 (471명 환자)를 대상으로 하였고, 이중 삼중음성 유방암군 87예를 최종 연구대상으로 하여 임상병리학적, 분자생물학적 자료를 분석하였다. 추적기간의 중앙값은 47.5개월 (1-100개월)이었다. 무재발생존율, 무병생존율, 유방암특이생존율, 그리고 전체생존율에 대한 단변량분석에는 Kaplan-Meier 방법을 적용하였고, log-rank test를 통하여 생존곡선을 산출하였다. 다변량분석에는 Cox proportional hazards model을 사용하여 분석하였다.

결과 :

총 476예에서 PCR-DHPLC-sequencing 방법으로 p53 유전자 돌연변이가 관찰된 것은 68예 (14.3%), 면역조직화학법으로 관찰된 것은 179예 (37.6%)였다. 총 476예 중 삼중음성 유방암군은 87예 (18.3%)이었고, 이중 22예 (25.3%)가 PCR-DHPLC-sequencing 방법으로, 51예 (58.6%)가 면역조직화학염색법으로 p53 돌연변이가 검출되었다. 삼중음성 유방암군에서 오직 연령만이 p53 유전자 돌연변이가 있는 군과 없는 군에서 통계적으로 유의한 차이를 보였고 (p = 0.002), 그밖에 다른 임상병리학적 항목들은 두 군간의 차이가 모두 통계적으로 유의하지 않았다 ($p > 0.05$). Exon 7이 삼중음성 유방암군에서 가장 호발하는 부위였고 (31.8%), 과오돌연변이가 가장 호발하는 형태였다 (59.1%). 삼중음성 유방암군에서 p53 과오돌연변이 유무에 따라 환자군을 나누었을 때, 단변량분석상 p53 과오돌연변이가 존재하는 군과 존재하지 않는 군사이에서 무재발생존율(relapse-free survival, RFS) (p = 0.048), 무병생존율(disease-free survival, DFS) (p = 0.060)이 통계적으로 유의한 차이를 나타내었고 유방암특이생존율(breast cancer-specific survival, BCSS) (p = 0.060)과 전체생존율(overall survival, OS) (p = 0.060)은 통계적으로 유의한 차이를 보이지는 않았으나 경향을 나타내었다. 다변량분석에서는 p53 과오돌연변이가 무재발생존율 (p = 0.040), 유방암특이생존율 (p = 0.048)과 전체생존율 (p = 0.048)에서 임상적으로 유의한 예후인자임을 나타내었고 무병생존율에 대해서는 예후인

자로서의 경향을 나타내었으나 통계적으로 유의하지는 않았다 ($p = 0.050$).

결론 :

본 연구는 PCR-DHPLC-sequencing으로 구성된 유전자 염기서열 분석법에 의하여 검출된 p53 과오돌연변이가 삼중음성 유방암군에서 무재발생존율 (RFS), 유방암특이생존율 (BCSS)과 전체생존율 (OS)에 대하여 임상적으로 의미있는 예후인자임을 제시하였다.

핵심단어 : 과오돌연변이, p53 유전자 돌연변이, PCR-DHPLC-sequencing, 예후, 삼중음성유방암