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The Predictive Role of E-cadherin and Androgen Receptor on *In Vitro* Chemosensitivity in Triple-negative Breast Cancer

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Objective: The purpose of this study was to evaluate the impact of various pathologic and biologic factors in triple-negative breast cancer (TNBC) on chemotherapy response using *in vitro* ATP-based chemotherapy response assay (ATP-CRA).
Methods: Forty-seven cases of TNBC were included. Immunohistochemical stains for androgen receptor (AR), p53, CD10, c-kit, CK5/6, vimentin, bcl-2, E-cadherin, Ki-67 and epidermal growth factor receptor were performed. *In vitro* ATP-CRA was used to analyze chemosensitivity for 5-fluorouracil (5-FU), docetaxel, doxorubicin, epirubicin, vinorelbine, gemcitabine, methotrexate (MTX), oxaliplatin and paclitaxel.
Results: The results showed that all cytotoxic agents demonstrated the trend that E-cadherin-expressing cases had a higher cell death rate than E-cadherin-negative cases. Particularly, vinorelbine showed statistical significance ($P = 0.004$). Cases with AR expression showed higher cell death rates than those without in 5-FU and MTX ($P = 0.012$ and 0.014, respectively).
Conclusions: E-cadherin and AR could be candidate predictive factors for chemotherapy response in TNBC. Further *in vivo* study is required to clarify their roles.

Key words: androgen receptor – breast cancer – chemosensitivity – E-cadherin – triple negative

INTRODUCTION

Human breast carcinoma is a heterogeneous tumor that is diverse in behavior, outcome and response to therapy. A substantial study to classify heterogeneous breast cancer has been performed. According to a gene-expression profile study, it has been divided into five subtypes with clinical implications (1–3): luminal A, luminal B, HER-2 overexpressing, normal breast-like and basal-like type (triple-negative phenotype). Previous DNA microarray and immunohistochemical (IHC) analyses have shown that 80–90% of triple-negative carcinomas are basal-like and have clinical behavior similar to the basal-like subtype (4). Triple-negative carcinomas account for ~15% of all invasive ductal carcinomas of no specific type (5). Conventional histopathological and molecular analyses of breast cancers have shown that basal-like tumors are often high grade (6), have areas of necrosis (7), may have a typical and atypical medullary phenotype (8) and have a distinct pattern of genetic alteration (6), including frequent TP53 mutation (1). The triple-negative phenotype [estrogen receptor (ER)−, progesterone receptor (PR)− and HER-2−] is increasingly used as a surrogate marker for basal-like breast cancer as those three stains are already routinely used in the clinical work-up of breast cancer (9). Although most basal-like tumors do not express ER, PR and HER-2, some may, and the overlap between basal-like and triple-negative phenotype is not complete (5). Triple-negative breast cancer (TNBC) is ER/PR negative for hormone therapy and HER-2/neu negative for trastuzumab therapy, so these targeted therapies are not very effective modalities. Standard treatment regimen for TNBC has not been established, and data about this issue are insufficient. Surface receptors such as epidermal growth factor receptor (EGFR), c-kit, protein kinase components of the mitogen-activated protein kinase pathway and protein kinase components of the protein kinase B pathway are suggested as potential therapy targets for TNBC, but these are under investigation. The present treatment modality for TNBC is combined chemotherapy, except for surgical treatment. Therefore, the detection of factors that affect chemoresponse in TNBC is essential, but the research on this has not been performed thoroughly.

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The purpose of this study was to evaluate the impact of various pathologic and biologic factors in TNBC on chemotherapy response using ATP-CRA.

PATIENTS AND METHODS

Patient Selection and Clinicopathologic Analysis

Forty-seven patients newly diagnosed with TNBC between January 2005 and December 2007 at Yongdong Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, were enrolled in this study. TNBC was confirmed by IHC stains. ER, PR and HER-2 stains were performed. ER and PR immunohistochemistry signal was evaluated using the Allred score (10). A score of 0–2 was considered negative and a score of 3–8 was considered positive. HER-2 staining was scored according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guideline (11) using the following categories: 0, no immunostaining; 1+, weak incomplete membranous staining in any proportion of tumor cells; 2+, complete membranous staining, either non-uniform or weak in at least 10% of tumor cells; and 3+, uniform intense membranous staining in >30% of tumor cells. Cases with 0–1+ were regarded as negative. Only ER-, PR- and HER-2-negative cases were included in this study. The study was approved by the Institutional Review Board of Yonsei University Severance Hospital, and written informed consent was obtained from all study participants. All patients were diagnosed as having invasive ductal carcinoma by a pathologist. All tissues were fixed in 10% buffered formalin and embedded in paraffin. All archival hematoxylin and eosin (H&E)-stained slides for each case were reviewed by two pathologists (J.S.K. and W.J.). Histologic grade was assessed using a modified Bloom–Richardson classification and nuclear grade was evaluated according to a modified Black's nuclear grade (1, low grade; 2, intermediate grade; and 3, high grade) (12). Histologic parameters were evaluated from H&E-stained slides. Clinicopathologic parameters evaluated in each tumor included patient age at initial diagnosis, sex and lymph node status.

IHC Staining

The antibodies used for immunohistochemistry in this study are shown in Table 1. All immunostainings were performed using formalin-fixed, paraffin-embedded tissue sections. Briefly, 5 μm-thick sections were obtained with a microtome, transferred into adhesive slides and dried at 62°C for 30 min. After incubation with primary antibodies, immunodetection was performed with biotinylated antimouse immunoglobulin, followed by peroxidase-labeled streptavidin using a labeled streptavidin biotin kit with 3,3′-diaminobenzidine chromogen as a substrate. The primary antibody incubation step was omitted in the negative control. Slides were counterstained with Harris hematoxylin. Normal breast tissues entrapped within the block and appropriate control tissues were used as positive controls.

Interpretation of IHC Stainings

All IHC markers were accessed by light microscopy. Scoring of immunostained slides was done according to the percentage of tumor cells exhibiting nuclear [androgen receptor (AR), Ki-67 and p53], nuclear and cytoplasmic (c-kit), cytoplasmic (CK5/6, vimentin and bcl-2), and membrane (E-cadherin and EGFR) staining. The IHC stain results of AR, p53, CD10, c-kit, CK5/6, vimentin, bcl-2, E-cadherin and EGFR were considered positive when >10% of tumor cell nuclei were stained. IHC stain results of Ki-67 were scored by counting the number of positively stained nuclei and expressed as a percentage of total tumor cells. These results were classified as follows: Group 1, <10%; Group 2, 10–30%; and Group 3, >30%. All samples were evaluated without knowledge of ATP-CRA results.

ATP-CRA Methodology

ATP-CRA was performed as described previously (13). Tumor tissues were stored in Hanks balanced salt solution (Gibco BRL, Rockville, MD, USA) containing 100 IU/ml of penicillin (Sigma, St Louis, MO, USA), 100 μg/ml of streptomycin (Sigma), 100 μg/ml of gentamicin (Gibco BRL), 2.5 μg/ml of amphotericin B (Gibco BRL) and 5% fetal bovine serum (FBS; Gibco BRL). When required, tissues were washed, quantitated, minced and then incubated with a mixture of dispase, pronase and DNase (Sigma) for 12–16 h at 37°C. Isolated cells were separated from tissue fragments by passing through a cell strainer (BD Falcon, Bedford, MA, USA). Tumor cells were separated from dead cells and red blood cells by ficoll (1.077 g/ml) gradient centrifugation at 400 g for 15 min. If a sufficient amount of cells were isolated, blood-derived normal cells were removed using

Table 1. Clone, dilution and source of antibodies used

Antibody	Clone	Dilution	Source
AR	AR441	1:100	Lab Vision Corp.
CK5/6	D5/16B4	1:100	DAKO, Denmark
E-cadherin	36B5	1:100	Novocastra, UK
p53	D0-7	1:100	Novocastra, UK
EGFR	EGFR.25	1:50	Novocastra, UK
Ki-67	MIB-1	1:150	DAKO, Denmark
bcl-2	3.1	1:50	Novocastra, UK
c-kit	Polyclonal	1:100	DAKO, Denmark
Vimentin	V9	1:150	DAKO, Denmark

AR, androgen receptor; CK5/6, cytokeratin 5/6; EGFR, epidermal growth factor receptor.

anti-CD45 antibody-conjugated magnetic beads (Miltenyi Biotech, Auburn, CA, USA) (14). The separated tumor cell preparation was suspended in IMDM (Gibco BRL) including 10% FBS. The cells were then diluted to a cell concentration between 2000 and 20 000 viable cells/100 μl for plating onto a 96-well ultra-low attachment microplate (Costar, Cambridge, MA, USA) with or without anti-cancer drugs and cultured for 48 h in the CO_2 incubator. The cytotoxic agents selected for assay were those commonly used in the treatment of breast cancer: 5-fluorouracil (5-FU), docetaxel, doxorubicin, epirubicin, vinorelbine, gemcitabine, methotrexate (MTX), oxaliplatin and paclitaxel.

Treated drug concentrations (TDCs) were determined by preliminary experiment, which exhibit scattered distribution of cell death from each specimen (15,16). The TDCs used were as follows: 5-FU, 50 μg/ml; docetaxel, 3.7 μg/ml; doxorubicin, 1.5 μg/ml; epirubicin, 1.2 μg/ml; paclitaxel, 8.5 μg/ml; vinorelbine, 0.18 μg/ml; gemcitabine, 16.9 μg/ml; MTX, 0.37 μg/ml; and oxaliplatin, 2.9 μg/ml. To measure ATP level, ATP in the cell lysate was reacted with luciferin and excessive luciferase (Roche, Mannheim, Germany) using Victor 3 multilabel counter (PerkinElmer, Boston, MA, USA). Excel-based raw data were analyzed by Report Maker version 1.1 (ISU ABXIS, Seoul, Korea). Briefly, the cell death rate for each drug was calculated as follows: cell death rate (%) = [1 − (mean luminescence in treated group/mean luminescence in untreated controls group)] × 100. To calculate the intra-assay mean coefficients of variation (CV), luminescence values of each specimen were measured three to six times in negative and positive control groups. We then determined whether measured values at 280 pg of ATP were higher than at 105 pg of ATP. If microorganism contamination was present, if there was an inadequate number of cells or if the intra-assay mean CV exceeded 30, the test concerned was considered a failure. If measured values in the untreated control group were lower than in the positive group (105 pg of ATP), the specimen was considered to have unacceptable viability.

Statistical Analysis

Statistical analyses were carried out using SPSS for Windows, version 12.0 (SPSS Inc., Chicago, IL, USA). Statistical significance of any differences observed for the expression of biologic markers in response to different cytotoxic drugs was calculated using *t*-test. Variables having more than three groups were analyzed with one-way ANOVA and multiple comparison test using Tukey b.

RESULTS

Patient and Tumor Characteristics

Patient and tumor characteristics are summarized in Table 2. Forty-seven patients were included in this study. All patients were women with a mean age of 49.7 ± 9.2 years (range, 28–70 years). Forty-two (89.3%) cases were invasive ductal carcinoma not otherwise specified (NOS). Medullary carcinoma in two (4.3%) cases, metaplastic carcinoma in two (4.3%) and tubular carcinoma in one (2.1%) were included. Out of 42 cases of invasive ductal carcinoma NOS, 15 (35.7%) patients showed the following histologic features of basal-like carcinoma: high nuclear and/or histologic grades, polygonal tumor cells with abundant eosinophilic or clear cytoplasm, distinct cytoplasmic border, squamoid appearance, large nests with central necrosis and intercellular eosinophilic materials.

Table 2. Characteristics of TNMC in this study

Parameters	Number of patients ($n = 47$) (%)
Histologic type	
Invasive ductal carcinoma, NOS	42 (89.3)
Medullary carcinoma	2 (4.3)
Metaplastic carcinoma	2 (4.3)
Tubular carcinoma	1 (2.1)
Age (years)	
<50	24 (51.1)
≥50	23 (48.9)
Histologic grade	
I	4 (8.5)
II	14 (29.8)
III	29 (61.7)
Nuclear grade	
1	3 (6.4)
2	11 (23.4)
3	33 (70.2)
Lymph node status	
No metastasis	32 (68.1)
Metastasis	15 (31.9)

NOS, not otherwise specified.

Histologic grade was scored as follows: grade I, 4 (8.5%) cases; grade II, 14 (29.8%); and grade III, 29 (61.7%). The number of cases of nuclear grade 1 was 3 (6.4%), nuclear grade 2 was 11 (23.4%) and nuclear grade 3 was 33 (70.2%). Fifteen (31.9%) cases showed axillary lymph node metastasis.

In Vitro Drug Sensitivity of Breast Cancer Cells by ATP-CRA

A list of the chemotherapeutic agents tested and their corresponding results are presented in Table 3. The cell death rate ranged from 0.0% to 92.9%. The results showed that paclitaxel had the narrowest range of cytotoxic effects (0.0–45.0%) with the lowest mean cell death rate (12.9%),

Table 3. *In vitro* chemosensitivity response of cultured breast cancer cell lines to a range of cytotoxic drugs according to the ATP-CRA

Cytotoxic drug	Rates of cell death (%)	
	Mean ± SD	Range
5-Fluorouracil	25.9 ± 12.6	0.0–59.4
Docetaxel	22.5 ± 14.5	0.0–52.7
Doxorubicin	28.8 ± 20.9	0.0–92.9
Epirubicin	32.1 ± 21.7	0.0–90.4
Paclitaxel	12.9 ± 12.6	0.0–45.0
Vinorelbine	31.8 ± 16.8	0.0–61.0
Gemcitabine,	19.9 ± 17.5	0.0–67.0
Methotrexate	21.6 ± 11.7	0.0–45.8
Oxaliplatin	34.5 ± 19.3	0.0–69.0

ATP-CRA, ATP-based chemotherapy response assay.

and doxorubicin had the widest range of cytotoxic effects (0.0–92.9%). The highest mean cell death rate was noted in oxaliplatin (34.5%).

Correlation Between ATP-CRA Results and Tumor Clinicohistologic Factors

Various clinocohistologic factors (histologic type and grade, nuclear grade and lymph node status) were analyzed to assess if there was any association between these factors and breast cancer cell response to cytotoxic agents. Table 4 shows the correlation between ATP-CRA results and tumor histologic factor. Cell death rate of all cytotoxic agents showed no significant difference among histologic subtypes. MTX revealed the highest cell death rates in histologic grade 1 ($P = 0.021$). Doxorubicin, epirubicin and oxaliplatin showed the trend of higher histologic grade demonstrating higher cell death rate. In contrast, 5-FU, paclitaxel and MTX showed that the lower the histologic grade, the higher the cell death rate. 5-FU, docetaxel, paclitaxel, vinorelbine and MTX showed the highest cell death rate in nuclear grade 1. In contrast, doxorubicin, epirubicin, gemcitabine and oxaliplatin showed the highest cell death rate in nuclear grade 3. Doxorubicin, epirubicin, gemcitabine and oxaliplatin revealed that the higher the nuclear grade, the higher the cell death rate. Vinorelbine and MTX showed that cases with higher nuclear grades demonstrated lower cell death rates. There was no significant difference in cell death rates between lymph node statuses in all chemotherapeutic agents.

Correlation Between ATP-CRA Results and Tumor Biologic Factors

The expression of various biologic factors (CK5/6, E-cadherin, p53, EGFR, vimentin, bcl-2, c-kit, Ki-67 and AR) as determined by immunohistochemistry was analyzed to identify any association between these factors and tumor cell death rates to cytotoxic agents. Table 5 demonstrates the correlation between ATP-CRA results and various tumor biologic factors. Seven (14.6%) cases of TNBC expressed CK5/6. CK5/6-expressed cases showed higher cell death rates than CK5/6-negative cases in all cytotoxic agents, except for docetaxel and oxaliplatin. E-cadherin was expressed in 42 (87.5%) cases. Figure 1 shows cell death rates in cytotoxic agents according to E-cadherin expression. All cytotoxic agents demonstrated the trend that E-cadherin-expressed cases had a higher cell death rate than E-cadherin-negative cases. In particular, vinorelbine showed statistical significance ($P = 0.004$). Expression of p53 was noted in 15 (31.3%) patients. There were inconsistent and variable cell death rates among cytotoxic agents without significant difference according to p53 status. EGFR was expressed in 20 cases (41.7%). In paclitaxel, EGFR-negative cases showed higher cell death rates than EGFR-expressed cases ($P = 0.042$). 5-FU and docetaxel demonstrated higher cell death rates in EGFR-negative cases without statistical significance. Seventeen (35.4%) patients expressed vimentin; however, there were inconsistent and variable cell death rates among cytotoxic agents without significant difference according to the status of vimentin expression. bcl-2 was expressed in seven (14.6%) cases. Except for doxorubicin, epirubicin and gemcitabine, all drugs showed higher cell death rates in bcl-2-expressed cases. c-kit was expressed in 12 (25.0%) patients. In 5-FU, cases with c-kit expression demonstrated higher cell death rate than those without ($P = 0.006$). Except for doxorubicin and gemcitabine, all drugs represented higher cell death rate in c-kit-expressed cases. Except for gemcitabine and oxaliplatin, all cytotoxic agents showed the highest cell death rate in group 3 for Ki-67; however, there was no statistically significant difference. AR was expressed in five (10.4%) cases. Figure 2 shows cell death rates in cytotoxic agents according to AR expression. In 5-FU and MTX, patients with AR expression showed higher cell death rates than those without ($P = 0.012$ and 0.014, respectively). TNBC was classified into 'basal-like carcinoma' and 'non-basal-like carcinoma' according to IHC results for CK5/6, EGFR and c-kit. Basal-like carcinoma was defined when the case showed positive expression of CK5/6 or EGFR or c-kit. Twenty-eight (59.6%) cases were basal-like carcinoma. Except for 5-FU and docetaxel, higher cell death rates were noted in basal-like carcinoma than non-basal-like carcinoma without statistical significance.

When five cases with specific subtype of medullary, metaplastic and tubular carcinoma were excluded, there was also significant association between chemosensitivity of vinorelbine and E-cadherin expression ($P = 0.004$), chemosensitivity of 5-FU and AR expression ($P = 0.010$) and chemosensitivity of MTX and AR expression ($P = 0.008$).

Table 4. Various tumor clinicohistological factors and ATP-CRA results

Parameter	5-FU (mean ± SD)	Docetaxel (mean ± SD)	Doxorubicin (mean ± SD)	Epirubicin (mean ± SD)	Paclitaxel (mean ± SD)	Vinorelbine (mean ± SD)	Gemcitabine (mean ± SD)	MTX (mean ± SD)	Oxaliplatin (mean ± SD)
Histologic type	$P = 0.950$	$P = 0.788$	$P = 0.798$	$P = 0.811$	$P = 0.796$	$P = 0.950$	$P = 0.392$	$P = 0.680$	$P = 0.936$
Invasive ductal carcinoma, NOS	25.70 ± 13.31	23.11 ± 14.78	29.51 ± 21.95	32.45 ± 22.62	13.44 ± 12.99	32.34 ± 17.12	21.46 ± 17.93	22.15 ± 11.97	35.06 ± 20.15
Medullary carcinoma	30.50 ± 4.38	16.00 ± 22.62	27.85 ± 4.31	26.25 ± 26.09	4.70 ± 0.00	26.25 ± 26.9	11.70 ± 5.09	17.20 ± 0.00	24.20 ± 0.00
Metaplastic carcinoma	24.25 ± 6.29	22.15 ± 2.89	26.00 ± 1.41	30.60 ± 15.27	6.25 ± 8.83	30.60 ± 15.27	7.45 ± 2.61	7.90 ± 0.00	27.30 ± 0.00
Tubular carcinoma	29.10 ± 0.00	11.00 ± 0.00	8.10 ± 0.00	26.30 ± 0.00	11.60 ± 0.00	26.30 ± 0.00	0.00 ± 0.00	22.50 ± 0.00	35.80 ± 0.00
Histologic grade	$P = 0.441$	$P = 0.284$	$P = 0.520$	$P = 0.667$	$P = 0.995$	$P = 0.567$	$P = 0.204$	$P = 0.021$	$P = 0.505$
I	33.72 ± 11.64	21.12 ± 19.58	18.10 ± 11.01	23.87 ± 11.53	13.52 ± 6.41	31.66 ± 12.45	5.37 ± 7.07	33.57 ± 9.75	22.06 ± 12.31
II	25.60 ± 10.72	17.57 ± 15.51	27.75 ± 20.88	30.77 ± 23.45	12.93 ± 14.04	35.88 ± 16.67	22.92 ± 13.66	25.11 ± 8.85	34.10 ± 24.53
III	24.99 ± 13.64	25.08 ± 13.22	30.83 ± 22.06	33.96 ± 22.19	12.80 ± 12.92	29.61 ± 17.45	20.59 ± 19.45	17.88 ± 11.58	36.19 ± 18.94
Nuclear grade	$P = 0.848$	$P = 0.398$	$P = 0.312$	$P = 0.223$	$P = 0.301$	$P = 0.893$	$P = 0.340$	$P = 0.203$	$P = 0.496$
1	28.80 ± 6.85	29.60 ± 18.80	19.10 ± 11.82	19.73 ± 14.01	20.90 ± 9.89	35.23 ± 18.58	5.46 ± 4.99	26.50 ± 5.65	27.10 ± 12.30
2	24.37 ± 12.01	18.00 ± 17.01	22.39 ± 24.01	25.00 ± 25.53	8.81 ± 12.08	33.07 ± 17.05	20.73 ± 14.34	26.26 ± 11.25	28.20 ± 22.43
3	26.19 ± 13.45	23.37 ± 13.36	31.87 ± 20.28	35.67 ± 20.45	13.55 ± 12.85	31.02 ± 17.10	21.06 ± 18.85	18.99 ± 11.80	37.16 ± 18.80
Lymph node status	$P = 0.683$	$P = 0.652$	0.647	$P = 0.084$	$P = 0.518$	$P = 0.266$	$P = 0.318$	$P = 0.376$	$P = 0.814$
No metastasis	26.44 ± 12.68	23.17 ± 15.48	27.86 ± 19.30	28.40 ± 20.68	13.71 ± 13.61	29.73 ± 16.83	18.22 ± 18.25	22.80 ± 12.88	33.91 ± 19.28
Metastasis	24.80 ± 13.00	21.09 ± 12.64	30.91 ± 24.79	40.15 ± 22.46	11.05 ± 10.20	36.10 ± 16.48	23.76 ± 15.85	18.98 ± 8.43	35.70 ± 20.21

5-FU, 5-fluorouracil; MTX, methotrexate.

Table 5. Various tumor biologic markers and ATP-CRA results

Parameter	Number ($n = 47$) (%)	5-FU (mean ± SD)	Docetaxel (mean ± SD)	Doxorubicin (mean ± SD)	Epirubicin (mean ± SD)	Paclitaxel (mean ± SD)	Vinorelbine (mean ± SD)	Gemcitabine (mean ± SD)	MTX (mean ± SD)	Oxaliplatin (mean ± SD)
CK5/6		$P = 0.441$	$P = 0.544$	$P = 0.901$	$P = 0.354$	$P = 0.831$	$P = 0.342$	$P = 0.767$	$P = 0.079$	$P = 0.775$
Positive	7 (14.6)	29.21 ± 14.31	19.22 ± 13.38	28.85 ± 15.20	37.84 ± 16.60	14.14 ± 13.08	37.70 ± 18.24	21.65 ± 17.43	29.98 ± 13.31	31.00 ± 15.44
Negative	39 (81.3)	25.12 ± 12.55	22.93 ± 14.96	27.80 ± 21.27	29.87 ± 21.28	13.01 ± 12.69	30.91 ± 16.61	19.47 ± 17.94	19.96 ± 11.14	34.48 ± 20.10
E-cadherin		$P = 0.121$	$P = 0.051$	$P = 0.165$	$P = 0.069$	$P = 0.095$	$P = 0.004$	$P = 0.151$	$P = 0.642$	$P = 0.054$
Positive	42 (87.5)	26.64 ± 12.58	23.66 ± 14.49	29.25 ± 20.63	32.79 ± 20.52	14.17 ± 12.68	34.69 ± 15.66	20.96 ± 17.89	21.68 ± 12.08	36.80 ± 19.18
Negative	4 (8.3)	16.27 ± 12.03	8.75 ± 8.91	14.40 ± 10.47	13.12 ± 13.64	3.12 ± 6.25	9.70 ± 8.88	7.60 ± 10.01	18.30 ± 9.49	16.75 ± 11.87
p53		$P = 0.587$	$P = 0.656$	$P = 0.724$	$P = 0.896$	$P = 0.589$	$P = 0.182$	$P = 0.212$	$P = 0.817$	$P = 0.068$
Positive	15 (31.3)	24.25 ± 8.07	23.77 ± 13.29	26.41 ± 9.83	30.50 ± 17.57	11.73 ± 11.31	37.26 ± 16.74	24.52 ± 20.07	20.70 ± 13.63	43.28 ± 19.48
Negative	31 (64.6)	26.46 ± 14.55	21.68 ± 15.43	28.71 ± 23.93	31.36 ± 22.29	13.92 ± 13.33	29.56 ± 16.66	17.52 ± 16.27	21.71 ± 11.17	29.56 ± 18.27
EGFR		$P = 0.059$	$P = 0.257$	$P = 0.927$	$P = 0.937$	$P = 0.042$	$P = 0.770$	$P = 0.235$	$P = 0.992$	$P = 0.698$
Positive	20 (41.7)	21.71 ± 12.53	19.55 ± 14.25	28.28 ± 20.26	31.36 ± 22.60	8.94 ± 11.22	32.95 ± 16.52	23.37 ± 18.73	21.39 ± 11.80	35.75 ± 20.20
Negative	26 (54.2)	28.85 ± 12.25	24.53 ± 14.87	27.71 ± 20.78	30.86 ± 19.53	16.58 ± 12.83	31.34 ± 17.58	17.06 ± 16.68	21.37 ± 11.11	32.89 ± 19.46
Vimentin		$P = 0.108$	$P = 0.685$	$P = 0.924$	$P = 0.622$	$P = 0.339$	$P = 0.704$	$P = 0.586$	$P = 0.331$	$P = 0.330$
Positive	17 (35.4)	21.79 ± 11.62	23.52 ± 13.88	27.58 ± 18.92	29.08 ± 21.26	10.85 ± 9.92	33.45 ± 17.73	21.68 ± 17.64	18.98 ± 11.01	38.45 ± 16.82
Negative	29 (60.4)	28.06 ± 13.00	21.68 ± 15.29	28.18 ± 21.43	32.25 ± 20.61	14.60 ± 13.97	31.30 ± 16.64	18.70 ± 17.93	23.00 ± 12.30	31.25 ± 21.05
bcl-2		$P = 0.095$	$P = 0.071$	$P = 0.176$	$P = 0.189$	$P = 0.252$	$P = 0.793$	$P = 0.211$	$P = 0.183$	$P = 0.912$
Positive	7 (14.6)	33.17 ± 9.39	31.55 ± 14.88	18.32 ± 8.47	21.57 ± 8.44	18.25 ± 10.81	34.27 ± 20.35	12.04 ± 14.06	27.96 ± 9.32	65.04 ± 20.61
Negative	39 (81.3)	24.41 ± 12.90	20.71 ± 14.18	29.69 ± 21.40	32.79 ± 21.80	12.25 ± 12.82	31.88 ± 16.76	21.20 ± 18.06	20.30 ± 11.94	33.95 ± 19.70
c-kit		$P = 0.006$	$P = 0.679$	$P = 0.486$	$P = 0.140$	$P = 0.280$	$P = 0.630$	$P = 0.775$	$P = 0.454$	$P = 0.051$
Positive	12 (25.0)	34.30 ± 12.40	20.99 ± 12.95	32.52 ± 25.18	40.18 ± 19.85	16.32 ± 15.96	33.91 ± 15.61	18.72 ± 16.01	24.21 ± 9.47	45.83 ± 19.33
Negative	35 (72.9)	23.04 ± 11.57	23.03 ± 15.17	27.57 ± 19.59	29.40 ± 21.93	11.69 ± 11.24	31.00 ± 17.40	20.42 ± 18.23	20.77 ± 12.42	30.54 ± 18.00
Ki-67		$P = 0.447$	$P = 0.986$	$P = 0.598$	$P = 0.331$	$P = 0.941$	$P = 0.972$	$P = 0.770$	$P = 0.112$	$P = 0.393$
Group 1	17 (36.2)	24.10 ± 10.19	22.55 ± 16.53	28.64 ± 21.10	32.61 ± 23.71	12.93 ± 13.76	31.20 ± 17.22	17.55 ± 17.96	18.16 ± 5.72	27.84 ± 19.46
Group 2	20 (42.6)	24.19 ± 20.59	22.45 ± 13.09	26.15 ± 18.24	27.69 ± 19.56	12.27 ± 11.33	31.76 ± 18.70	21.77 ± 18.02	19.40 ± 16.56	39.04 ± 17.33
Group 3	10 (21.2)	29.07 ± 9.10	22.56 ± 15.20	34.52 ± 26.52	40.31 ± 22.10	14.04 ± 14.14	33.01 ± 13.17	20.58 ± 17.18	27.01 ± 12.35	33.27 ± 23.03
AR		$P = 0.012$	$P = 0.859$	$P = 0.250$	$P = 0.250$	$P = 0.190$	$P = 0.259$	$P = 0.096$	$P = 0.014$	$P = 0.431$
Positive	5 (10.4)	39.16 ± 8.68	23.62 ± 21.54	18.54 ± 10.52	21.50 ± 11.20	19.94 ± 12.42	40.90 ± 13.18	7.62 ± 7.19	34.95 ± 9.55	23.90 ± 16.82
Negative	42 (87.5)	24.34 ± 12.19	22.38 ± 13.83	30.06 ± 21.65	33.42 ± 22.41	12.04 ± 12.52	30.79 ± 16.98	21.46 ± 17.87	19.96 ± 10.97	35.22 ± 19.47
IHC subtype		$P = 0.762$	$P = 0.213$	$P = 0.965$	$P = 0.468$	$P = 0.814$	$P = 0.404$	$P = 0.469$	$P = 0.466$	$P = 0.422$
Basal-like	28 (59.6)	25.45 ± 14.14	20.32 ± 14.30	28.95 ± 22.50	34.07 ± 22.50	13.26 ± 13.68	33.45 ± 16.09	21.53 ± 18.22	22.79 ± 12.34	36.90 ± 19.59
Non-basal-like	19 (40.4)	26.61 ± 10.45	25.74 ± 14.63	28.66 ± 19.11	29.32 ± 20.82	12.35 ± 11.11	28.74 ± 18.20	17.71 ± 16.71	19.81 ± 10.85	31.15 ± 19.06

IHC, immunohistochemistry.

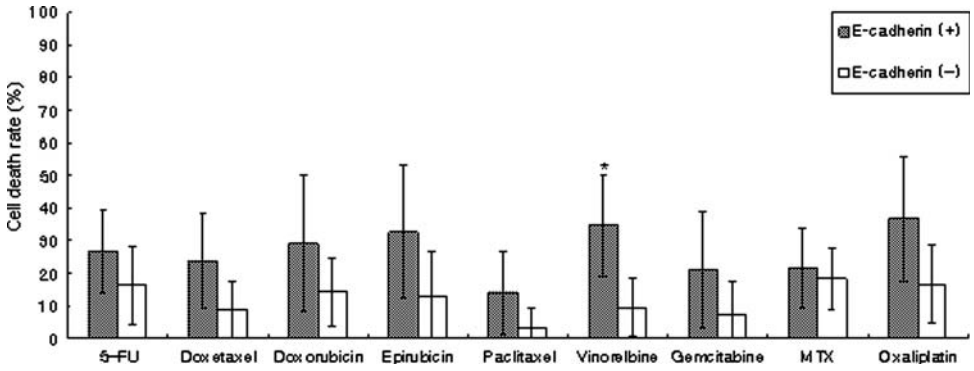

Figure 1. Comparison of cell death rates in various cytotoxic agents according to E-cadherin expression. 5-FU, 5-fluorouracil; MTX, methotrexate. *$P < 0.05$.

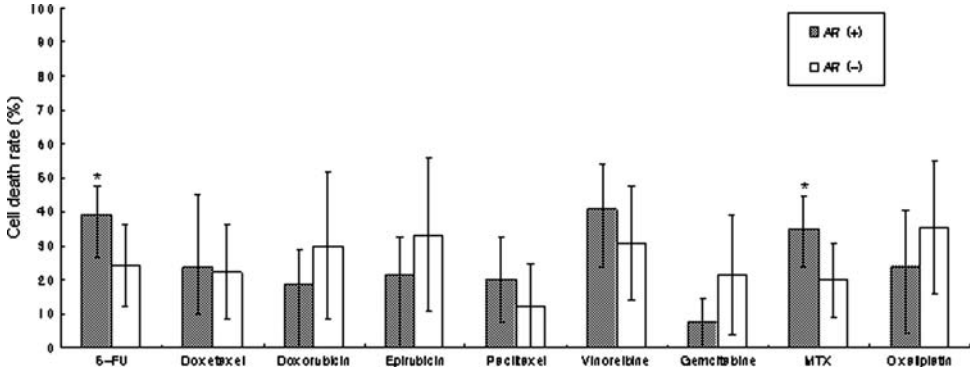

Figure 2. Comparison of cell death rates in various cytotoxic agents according to androgen receptor expression. AR, androgen receptor; *$P < 0.05$.

DISCUSSION

Studies on detecting factors that affect chemosensitivity in breast cancer have been extensively performed through neoadjuvant chemotherapy. Among the known predictive factors, those that apply to TNBC are ER negativity (17) and high expression of Ki-67 (18). In previous studies, preoperative paclitaxel and doxorubicin chemotherapy showed that complete pathologic response rate of basal-like cancer was up to 45% (19), and neoadjuvant anthracycline-based chemotherapy demonstrated that the clinical response rate of basal-like cancer was up to 85% (20). However, studies on predictive factors for chemotherapy response in TNBC have not been performed. In this study, we investigated various pathologic and biologic factors to evaluate the effect on chemosensitivity in 47 cases of TNBC by using *in vitro* ATP-CRA. The results showed that E-cadherin and AR could be candidate predictive factors in TNBC.

E-cadherin is a transmembrane glycoprotein synthesized by the CDH1 gene located in chromosome 16q22.1, and its role is thought to be in cell proliferation, invasion and a metastasis suppressor (21). E-cadherin inactivation by mutation, loss of heterozygocity or methylation is characteristic of invasive lobular carcinoma in the breast. Loss of E-cadherin is related to larger tumor size, higher tumor grade, and higher prevalence and metastasis in breast cancer (22,23). Four (8.3%) cases of TNBC showed loss of E-cadherin in this study. A previous study showed that >40% of basal-like and triple-negative phenotypes showed loss of E-cadherin among non-lobular breast carcinomas, which was higher than that of other phenotypes (21). The exact mechanism of E-cadherin loss in basal-like and triple-negative phenotypes is not known; however, loss of E-cadherin is suggested to be related to invasion and metastasis pattern, which is characteristic of basal-like and triple-negative phenotypes (21). In this study, TNBC with E-cadherin expression showed higher chemoresponse than TNBC without E-cadherin expression in all chemotherapeutic agents, particularly vinorelbine ($P = 0.004$). The possible mechanism that TNBC showing loss of E-cadherin represented decreased chemosensitivity is thought to be through epithelial-to-mesenchymal transition. Down-regulation of E-cadherin expression is known to a hallmark of epithelial-to-mesenchymal transition (24). Epithelial-to-mesenchymal transition means that epithelial cells are dedifferentiated to fibroblastoid migratory cells, and these mesenchymal cells are thought to be less sensitive to chemotherapy (25). TNBC is reported to express vimentin, which is a representative marker of mesenchymal cells (26), and all four cases with loss of E-cadherin expressed vimentin. These findings support the above hypothesis. An *in vitro* study showed that expression of E-cadherin decreased bcl-2 expression and increased sensitivity to etoposide-induced

apoptosis (27). This could be another possible mechanism of resistance to chemotherapeutic drugs due to loss of E-cadherin. However, another *in vitro* study demonstrated that tumor cells with E-cadherin expression showed lower sensitivity to cisplatin than tumor cells without E-cadherin expression, and that there was no difference in etoposide and 5-FU (28). Therefore, we cannot conclude the effect of E-cadherin on chemotherapy response yet.

This study demonstrated that patients with AR expression showed higher cell death rates than those without in 5-FU and MTX ($P = 0.012$ and 0.014, respectively). The role of AR in breast cancer in breast carcinogenesis, and progression is still uncertain. It has been known that AR shows expression in ~70–90% of invasive breast cancer (29), lobular carcinoma (30), BRCA-mutated tumor (31) and mammary Paget's disease (32). It is known that there is a relationship between AR and ER/PR status (30,33–36), but a significant percentage of tumors are positive for AR and negative for ER and PR (34). This finding represents the independent expression of AR in human breast cancer. However, there were divergent data about biologic and clinical signification in AR of breast cancer. Univariate analysis demonstrated that AR showed prognostic power along with ER, tumor size, tumor grade, lymph node status and high level of Ki-67 (29). In ER-negative tumors, AR-positive patients exhibited significantly better disease-free survival than AR-negative tumors (37), and in invasive ductal carcinoma, AR-positive tumors have been associated with a low or intermediate histologic grade (30,34,36–38). However, there was a report that breast cancer with the AR gene or AR protein showed an increased tendency of axillary lymph node metastasis (39). In triple-negative carcinoma, especially lymph node-positive, as expression of AR was lost, there was an increased incidence of high nuclear grade, development of recurrence and distant metastasis. Therefore, AR could play a role as a prognostic factor in triple-negative carcinoma (40). However, a study of AR's role as a predictive factor for chemotherapy in breast cancer, particularly TNBC, has not been performed to our knowledge. In ER, other hormone receptor-like AR, breast cancer with lower ER expression was reported to show higher response to chemotherapy (41), but in other reports, ER-rich tumors showed higher response to chemotherapy (42). AR expression rate in this study was 10.4%, which is compatible with that of previous reports that ranged from 13% to 32% (40,43). The reason why TNBC with AR expression shows increased chemosensitivity in 5-FU and MTX is not known. However, a possible mechanism is thought to be through the AR signaling pathway. The AR signaling pathway is activated by the androgen-dependent and -independent pathway (44). We thought that 5-FU and MTX could inhibit breast cancer cell proliferation through the AR signaling pathway in TNBC with AR expression.

The limitation of this study is that it is an *in vitro* study, so it could not be concluded that these results would apply to real patients. However, in a previous breast cancer study, the chemoresponse results of *in vitro* ATP-CRA were consistent with those of real patients at ~85% (45). A study on real patients has its shortcomings as it cannot investigate the chemoresponse of a single agent due to combined chemotherapy regimens, but an *in vitro* study can.

In conclusion, E-cadherin and AR could be candidate predictive factors for chemotherapeutic drugs in TNBC. Further *in vivo* study is required.

Conflict of interest statement

None declared.

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