Table 2
Adverse effects of TJ chemotherapy

Adverse effect	Grade (NCI-CTC)			
	1	2	3	4
Leukopenia	0	0	2	0
Anemia	0	3	1	1
Neutropenia	3	0	1	1
Nausea/vomiting	2	1	0	0
Peripheral neurotoxicity	3	1	1	0
Cardiotoxicity	0	1	0	0
Alopecia	0	6	–	–

NCI-CTC: National Cancer Institute—Common Toxicity Criteria. (NCI-CTC Version 2.0, Jan. 30, 1998).

Five of the six cases had measurable disease at the time paclitaxel and carboplatin chemotherapy was started. Cases 1, 4, and 5 had measurable regions in the lungs, Case 2 in the lungs and liver, and Case 6 in the pelvic cavity and liver. All regions were measured by spiral computed tomography (CT) and/or magnetic resonance imaging (MRI) before initial administration of paclitaxel and carboplatin chemotherapy.

The combination of paclitaxel (175 mg/m^2 over 3 h) and carboplatin (dosed at AUC 6, according to the Calvert formula) was given intravenously every 3 weeks. All patients received pretreatment medications designed to decrease allergic reactions to paclitaxel. The pretreatment regimen consisted of the following: (1) dexamethasone 20 mg intravenously 60 min before paclitaxel, (2) diphenhydramine 50 mg orally 60 min before paclitaxel, and (3) ranitidine 50 mg intravenously 60 min before paclitaxel.

Response was assessed according to Response Evaluation Criteria in Solid Tumors [20]. Complete response (CR) was defined as the disappearance of all target lesions with confirmation at 4 weeks. Partial response (PR) was at least 30% reduction in the sum of the longest diameter of target lesions, taking as reference the baseline study; this also was confirmed at 4 weeks. Progressive disease (PD) was defined at least 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since treatment started or new lesions appeared. Patients who did not meet any of these criteria were considered to have stable disease (SD). Evaluation of overall response was determined by achievement with both target and nontarget lesions. Progression-free interval (PFI) was defined as the date of study entry to the date of reappearance or increased parameters of disease or to the day of last contact. Overall survival was the observed length of life from study entry to death or to the day of last contact.

Pretreatment evaluation for chemotherapy included the following: white blood count > 3000/μl, platelet count > 100,000/μl, blood urea nitrogen level < 30 mg/dl, serum creatinine < 1.5 mg/dl, creatinine clearance > 50 ml/min,

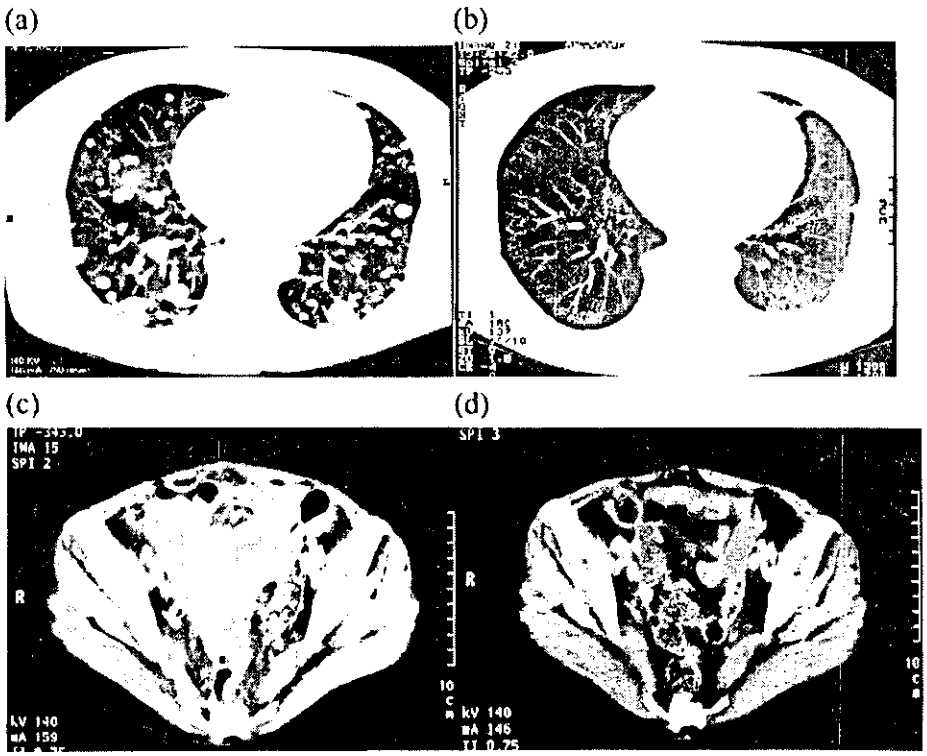

Fig. 1. (a) Pretreatment CT of the lungs and mediastinum for Case 5 shows multiple metastases. (b) CT taken after the third course of paclitaxel and carboplatin at a similar level to the scan shown in (a) shows complete disappearance of the metastatic tumors. (c) Pelvic CT for Case 6 shows 11 × 7 cm of recurrent tumor before chemotherapy began. (d) CT taken after the 10th course of paclitaxel and carboplatin at a similar level to the scan shown in (c) shows complete disappearance of the tumor.

serum bilirubin < 1.5 times normal, AST and ALT < 3 times normal, serum albumin > 3 g/dl, normal electrocardiographic pattern, normal spirometry, and a GOG performance status of 0–2. Written informed consent was obtained from all patients before initiation of treatment. Hematologic indexes were monitored weekly. Patients were removed from this study if there was clear evidence of disease progression or severe toxicity. Toxicities were evaluated according to the National Cancer Institute—Common Toxicity Criteria [21].

Results

For the total of six patients, age ranged from 47 to 63 years with a median of 56 years. The median number of chemotherapy courses administered was 9 (range, 4–14). Adverse effects for the six patients during paclitaxel and carboplatin treatment are summarized in Table 2. Grades 3 and 4 hematologic toxicities occurred in four and two patients, respectively, but all of them were overcome by adequate treatment with granulocyte colony-stimulating factor and blood transfusions. One case developed grade 3 peripheral neurotoxicity, but this adverse event did not stop the protocol. No cases developed hypersensitivity reactions. There were not any treatment delay through all period.

In the five patients with measurable disease, there were four complete responses (CR) and one progressive disease (PD) as measured by spiral CT scan and, in some cases, by MRI. Two representative CT scans are shown in Fig. 1. The overall response rate was 4/5, and the CR rate was the same.

Two patients (Case 4 and 5) experienced recurrence. Case 4 had recurrent regions in the lungs and kidneys after a 16-month progression-free interval (PFI). She was treated with docetaxel and carboplatin because of severe neurotoxicity after the 10th course of paclitaxel and carboplatin chemotherapy. However, metastatic regions grew again during chemotherapy, and docetaxel and carboplatin were stopped. She was alive with disease 7 months after the end of the second-line therapy. Case 5 had recurrent tumors in her lungs after a 20-month PFI. She was treated with radiation therapy, resulting in CR again, and she was alive without disease 10 months after the beginning of her second CR.

The median PFI of all six cases was 18 months (range, 0–32) and the median overall survival was 25 months (range, 6–32).

Discussion

To the best of our knowledge, this is the first report in which combination chemotherapy with paclitaxel and carboplatin has been used against uterine CS. Historically, numbers of chemotherapeutic agents have been utilized in uterine CS, and some of these are summarized in Table 3. In the present study, we obtained a higher overall response rate and CR rate than was observed in previous studies, although our number of cases is small for the purposes of statistical analysis, and none of six patients received prior radiation therapy.

Two prospective studies have shown higher response rates with combination chemotherapy than with single usage of ifosphamide, with the specific combinations of ifosphamide plus cisplatin, 54% in Gynecologic Oncology Group (GOG) study [7], and a combination of ifosphamide, doxorubicin, and cisplatin, 56% in the European Organization for Research ant Treatment of Cancer Gynaecological Cancer Group (EORTC) 55923 [10]. The median overall survival with measurable disease, 26 months, obtained in the EORTC study was similar to the value observed in this study, 25 months. Despite the small scale and short follow-up period of the present study, the observed 4 CR/5 patients with measurable disease and 25 months of median survival seem to be very promising. Additional phase II studies are needed to confirm and extend the results.

Table 3
Responses of chemotherapeutic trials in uterine carcinosarcoma

Author	*N*	Chemotherapeutic regimen	Response rate	CR rate
Sutton et al. [6][a]	28	IFX	32%	18%
Thigpen et al. [8][a]	63	CDDP	19%	8%
Curtin et al. [9][a]	44	Paclitaxel	18%	9%
Piver et al. [23][b]	23	CPA + VCR + ADR + DTIC	23%	12%
Hannigan et al. [24][b]	74	VCR + ACT-D + CPA	29%	13%
Currie et al. [25][a]	32	HU + DTIC + VP-16	16%	6%
van Rijswijk et al. [10][a]	32	CDDP + ADR + IFX	56%	34%
Omura et al. [4][b]	146	ADR vs. ADR + DTIC	16% vs. 24%	6% vs. 11%
Muss et al. [5][b]	52	ADR vs. ADR + CPA	19% vs. 19%	4% vs. 8%
Sutton et al. [7][a]	92	IFX vs. IFX + CDDP	36% vs. 54%	24% vs. 31%

N: number of patients with measurable disease.
CPA: cyclophosphamide; VCR: vincristine; ADR: doxorubicin; DTIC: dacarbazine.
ACT-D: actinomycin; IFX: ifosfamide; CDDP: cisplatin; HU: hydroxyurea; VP-16: etoposide.
[a] Only uterine carcinosarcoma.
[b] Uterine sarcoma.

The GOG is currently running two clinical trials related to CS of the uterus [22]. One is a phase III randomized study comparing the combination of ifosfamide and paclitaxel with single-agent ifosfamide for patients with advanced or recurrent CS of the uterus (GOG161). The other is a phase III randomized study comparing whole abdominal radiotherapy with combination chemotherapy (ifosfamide and cisplatin) in patients with optimally debulked CS of the uterus (GOG150). If the effectiveness of paclitaxel and carboplatin against uterine CS is confirmed by future studies, we can design a phase III randomized control study comparing paclitaxel and carboplatin with an advantaged therapy identified through the previously mentioned GOG studies.

Acknowledgments

This work was supported, in part, by a grant-in-aid from the Kurokawa Cancer Research Foundation, by a grant-in-aid for scientific area on priority area from the Ministry of Education, Science and Culture, and by a grant-in-aid from the Ministry of Health and Welfare, Japan.

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Jpn J Clin Oncol 2004;34(1)43–45

Clinical Trial Note

Feasibility Study of Neoadjuvant Chemotherapy Followed by Interval Cytoreductive Surgery for Stage III/IV Ovarian, Tubal and Peritoneal Cancers: Japan Clinical Oncology Group Study JCOG0206

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Received October 7, 2003; accepted December 3, 2003

A feasibility study was started in January 2003 on neoadjuvant chemotherapy (NAC) followed by interval cytoreductive surgery (ICS) and postoperative chemotherapy for stage III/IV müllerian carcinomas such as ovarian, tubal and peritoneal carcinomas. The purpose is to assess the safety and efficacy of the treatment starting with NAC and also to know whether we can accurately diagnose these advanced carcinomas by imaging studies, cytologic findings and tumor markers without staging laparotomy or laparoscopy. Fifty-six patients with advanced müllerian carcinomas will be recruited to the study. After confirmation of diagnosis by laparoscopic inspection and biopsies, patients undergo four cycles of chemotherapy as NAC, followed by ICS and an additional four cycles of post-surgical chemotherapy. The primary endpoint is proportion of clinical complete remission after accomplishment of the protocol treatment, while the major secondary endpoint is positive predictive value of diagnosis before laparoscopy regarding tumor origin, histology and stage. Based on the results of this study, we will conduct a phase III study to compare the treatment starting with NAC and primary cytoreductive surgery followed by post-surgical chemotherapy.

Key words: ovarian neoplasms – laparoscopy – neoadjuvant therapy – interval cytoreductive surgery

INTRODUCTION

Prognosis of patients with advanced epithelial ovarian, tubal and peritoneal carcinomas is known to be poor. Even using platinum compound regimens, the 5-year survival rate of stage III/IV ovarian cancer is still around 20% (1). The current standard treatment for advanced ovarian cancer is primary cytoreductive surgery followed by post-surgical chemotherapy. However, optimal cytoreduction in primary surgery can be achieved only in 40% of stage III/IV ovarian cancer patients (2). An alternative to primary surgical cytoreduction in patients with unresectable bulky tumors or poor performance status is the use of chemotherapy in the neoadjuvant setting. Recent retrospective analyses (3–6) have revealed that progression-free and overall survival were comparable between patients treated with neoadjuvant chemotherapy (NAC) followed by interval cytoreductive surgery (ICS) and those treated by primary cytoreductive surgery, though the former group was older and had a poorer performance status. Phase II and III trials have not been performed on the role of neoadjuvant-setting treatment for advanced ovarian, tubal and peritoneal cancers. Therefore, we started a phase II study to assess the safety and efficacy of NAC followed by ICS and post-surgical chemotherapy before comparing with the current standard treatment including primary cytoreductive surgery in randomized controlled trial. Neoadjuvant setting has the advantage of earlier treatment start and lower invasiveness. However, according to the current general rules for the management of ovarian cancer, it is neces-

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sary to confirm the origin, histology and stage before starting treatment by staging laparotomy or laparoscopy. Thus, we also determine whether we can omit the 'extra procedure' of staging laparotomy or laparoscopy before the neoadjuvant-setting treatment in the majority of patients with advanced ovarian, tubal or peritoneal cancer.

The study protocol was designed by Gynecologic Cancer Study Group (GCSG) of the Japan Clinical Oncology Group (JCOG), approved by the Clinical Trial Review Committee of JCOG on December 6, 2002, and activated on January 14, 2003.

PROTOCOL DIGEST OF THE JCOG0206

PURPOSE

The purposes are to assess the safety and efficacy of the treatment starting with NAC with paclitaxel and CBDCA for phase III study, comparing NAC therapy with current standard procedure, and to know whether we can accurately diagnose these advanced carcinomas by imaging studies, cytologic findings and tumor markers without staging laparotomy or laparoscopy.

STUDY SETTING

A multi-institutional (26 centers) non-randomized phase II trial.

RESOURCES

Health Sciences Research Grants for Clinical Research for Evidenced Based Medicine and Grants-in Aid for Cancer Research (nos 14S-4, 14-12), from the Ministry of Health, Labor and Welfare, Japan.

ENDPOINTS

Primary endpoint is proportion of clinical complete remission (%cCR) among all stage III or IV müllerian carcinoma confirmed by laparoscopic inspection and histopathology of biopsy specimens. Clinical complete remission is defined as disappearance of all lesions by computed tomography (CT) or magnetic resonance imaging (MRI), no pleural effusions by chest radiography and normal serum CA125 level (<20 U/ml) after completion of the protocol treatment.

Secondary endpoints are as follows: (i) positive predictive value (PPV) of pre-laparoscopic diagnosis concerning the origin and histology—proportion of the patients diagnosed as müllerian carcinoma by laparoscopic inspection and histopathology of biopsy specimen among those diagnosed by pre-laparoscopic findings; (ii) PPV of prelaparoscopic diagnosis concerning clinical stage—proportion of the patients diagnosed as stage III or IV by laparoscopic inspection among those diagnosed by pre-laparoscopic findings; (iii) PPV of overall pre-laparoscopic diagnosis—proportion of the patients diagnosed as stage III or IV müllerian carcinoma by laparoscopic inspection and histopathology of biopsy specimen among those diagnosed by pre-laparoscopic findings.

Other secondary endpoints are: (iv) response rate to NAC among patients whose clinical diagnosis is confirmed by laparoscopy; (v) proportion of patients who received ICS among patients whose clinical diagnosis is confirmed by laparoscopy; (vi) progression-free survival among patients whose clinical diagnosis is confirmed by laparoscopy; (vii) operative morbidity among all enrolled patients; (viii) adverse events among all enrolled patients: and (ix) overall survival among all enrolled patients.

ELIGIBILITY CRITERIA

INCLUSION CRITERIA

The study subjects are patients diagnosed as stage III or IV müllerian carcinoma by pre-laparoscopic clinical findings including imaging studies (CT, MRI or ultrasonography) and cytology of ascites, pleural effusions or fluids obtained by tumor centesis. Malignancies of other origins, such as breast and digestive tract, should be excluded by endoscopy, opaque enema or ultrasonography when these malignancies are suspected from symptoms, physical examination or imaging diagnosis. To rule out malignancy of digestive tract origin, criteria for tumor markers are set to be CA125 >200 U/ml and CEA <20 ng/ml.

Further inclusion criteria are: (i) clinically deemed to be a candidate for debulking surgery without evidence of brain, bone, bone marrow metastases, multiple lung or multiple liver metastases; (ii) presence of at least one measurable lesion; (iii) previously untreated for these malignancies and no history of treatment with chemotherapy nor radiotherapy even for other diseases; (iv) age 20–75 years; (v) Eastern Cooperative Oncology Group (ECOG) performance status of 0–3; (vi) adequate bone marrow, hepatic, renal, cardiac and respiratory functions; and (vii) written informed consent.

EXCLUSION CRITERIA

These are: (i) synchronous or metachronous (within 5 years) malignancy other than carcinoma in situ; (ii) pregnant or nursing; (iii) severe mental disorders; (iv) systemic and continuous use of steroidal drugs; (v) active infections; (vi) uncontrolled hypertension; (vii) diabetes mellitus, uncontrolled or controlled with insulin; (viii) history of cardiac failure, unstable angina, myocardial infarction within 6 months prior to the registration; (ix) liver cirrhosis or bleeding tendency contraindicating debulking surgery; (x) intestinal occlusion necessary for surgical treatment; and (xi) hypersensitivity to alcohol.

TREATMENT METHODS

DIAGNOSTIC LAPAROSCOPY

After enrolment, diagnostic laparoscopy is performed within 2 weeks. To confirm pre-laparoscopic clinical diagnosis of origin, histology and stage, inspection of peritoneal cavity and biopsy from the main tumor or metastatic tumors are per-

formed. Resection of any organs or tumors attempting to reduce tumor volume is not allowed.

NEOADJUVANT CHEMOTHERAPY (NAC)

Four cycles of combination of paclitaxel (175 mg/m^2, day 1) and carboplatin (AUC = 6, day 1) are administered every 3 weeks. NAC is initiated within 1 week after laparoscopy.

INTERVAL CYTOREDUCTIVE SURGERY (ICS)

ICS is performed in 4–7 weeks after administration of the fourth cycle of NAC unless disease progression occurs during NAC. Standard procedures of ICS consist of total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and maximal debulking of metastatic tumors. Systematic pelvic and/or aortic lymphadenectomies are allowed, but not included in standard procedures.

POST-SURGICAL CHEMOTHERAPY

An additional four cycles of chemotherapy (same regimen as NAC) is administered (eight cycles of chemotherapy in total). Post-surgical chemotherapy is initiated within 3 weeks after ICS.

STUDY DESIGN AND STATISTICAL METHODS

The study is planned as a single-stage safety and efficacy study. Sample size calculation was primarily based on binominal test for the primary endpoint, %cCR. Forty-four eligible patients are required when expected %cCR of 40% and an acceptable lowest %cCR of 20% with alpha error level of 0.05 and beta error level of 0.1. Additionally, PPV is to be confident enough to omit laparoscopy before NAC in the following phase III study. It is not possible to use sensitivity or specificity to evaluate accuracy of clinical diagnoses, because laparoscopy is performed only in patients diagnosed as stage III/IV müllerian carcinomas by clinical findings in this study setting. Thus, Bayesian monitoring PPV is planned, which requires 56 patients to have the 10% or lower Bayesian posterior probability that PPV is <90% in case of three false positive patients assuming prior distribution of beta (9,1). The target sample size was determined to be 56, which also can be expected sufficient for primary endpoint. The planned accrual period is 1 year and the follow-up period is set as 3 years after the completion of accrual.

STUDY MONITORING

In-house interim monitoring is performed by the JCOG Data Center to ensure data submission, patient eligibility, protocol compliance, safety and on-schedule study progress according to the JCOG standard procedures. The monitoring reports are submitted to the JCOG Data and Safety Monitoring Committee every 6 months.

PARTICIPATING INSTITUTIONS

Hokkaido University, Sapporo Medical University, Tohoku University, University of Tsukuba, Gunma Prefectural Cancer Center, Shinshu University, National Defense Medical College, Saitama Cancer Center, National Cancer Center Hospital, The Jikei University School of Medicine, Cancer Institute Hospital, University of Tokyo, Juntendo University, Nagaoka Red Cross Hospital, Aichi Cancer Center, National Nagoya Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, Kinki University, Niigata Cancer Center, Kure National Hospital (Chugoku District Cancer Center), National Shikoku Cancer Center, National Kyushu Cancer Center, University of Kurume, Kyushu University, Saga Medical School and Kagoshima City Hospital.

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Expression of Insulin-Like Growth Factor 1 Receptor in Primary Breast Cancer: Immunohistochemical Analysis

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Insulin-like growth factor-1 receptor (IGF-1R) has been implicated in regulation in tumor growth. The results of previous studies performed by radioimmunoassay are conflicting, and the prognostic significance of IGF-1R expression in primary breast cancer is still controversial. IGF-1R expression was evaluated in formalin-fixed, paraffin-embedded tissue of 210 primary breast cancer patients by using anti-IGF-1R antibody. The clinicopathologic variables and 5-year disease-free survival were studied, and their correlations between IGF-1R expressions were investigated. IGF-1R overexpression was observed in 43.8% of tumors. IGF-1R overexpression had no correlation with prognosis or with other clinicopathologic parameters, such as age, tumor size, nodal status, histologic grade, hormone receptor status, and human epidermal growth factor 2 status. Though its prognostic value in breast cancer is limited, immunohistochemical evaluation of IGF-1R by using this monoclonal antibody may be useful in translational research using archived material. HUM PATHOL 35:1537-1542.

Key words: **Insulin-like growth factor 1 receptor, immunohistochemistry, primary breast cancer, prognostic marker.**

Abbreviations: **IGF-1R, insulin-like growth factor-1 receptor; IGFBPs, IGF-binding proteins; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; IHC, immunohistochemistry; DFS, disease-free survival; RIA, radioimmunoassay.**

IGF-1R is a glycosylated heterotetramer composed of 2 extracellular α-subunits and β-subunits that have intrinsic tyrosine kinase activity with 70% homology to the insulin receptor.[1] IGF-1R mainly mediates the effect of insulin-like growth factors (IGFs), which are potent mitogens that regulate cell proliferation, differentiation, and protection from apoptosis.[2] The clinical and epidemiologic data suggest that the levels of IGF-1 or IGF binding proteins (IGFBPs) in the serum are related to the risk of solid tumors such as breast, prostate, endometrial, ovarian, and colon cancer.[3]

IGF-1R has been found to be significantly expressed and highly activated in breast cancer, and its prognostic and predictive value in clinical samples are of interest.[4-8] There are several methods to measure IGF-1R expression: radioimmunoassay, Western blotting, and immunohistochemistry (IHC). Immunohistochemical evaluation is the most simple and the easiest to perform. To date, there are several commercially available anti-IGF-1R antibodies, but there are no established scoring methods for IGF-1R expression in formalin-fixed, paraffin-embedded tissue. We herein report the prognostic significance of IGF-1R overexpression as determined by IHC on archive materials of consecutive primary breast cancer patients when evaluated by the intensity of membrane staining. We also investigated its correlation with various clinicopathologic factors.

MATERIALS AND METHODS

Patients

This study was performed on 276 consecutive primary breast cancer patients who underwent surgery or biopsy at National Cancer Center Hospital from January to December 1997. From the cases, 268 paraffin-embedded formalin fixed tissues were obtained. Thirteen stage IV breast cancer patients, 9 patients with malignancy of other origin, 7 metachronous bilateral breast cancer patients, 4 synchronous breast cancer patients, and cases impossible for evaluation in invasive component such as ductal carcinoma in situ were excluded from analysis. Thus immunohistochemical staining was performed on 210 invasive carcinomas.

Pathology

Tumor size, number of axillary lymph node metastasis, histologic type, and histologic grade according to Nottingham combined histologic grading were noted.

Immunohistochemistry

IHC was performed for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2), and IGF-1R on 4-μm-thick serial sections from formalin-fixed, paraffin-embedded tissue.

Monoclonal antibodies 1D5 (DAKO) and 1A6(DAKO) were used for ER and PR IHC, respectively, according to the recommended staining protocol by the manufacturer. It was scored to be positive when $\geq$10% of the cancer cells were

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0046-8177/$—see front matter

doi:10.1016/j.humpath.2004.09.005

TABLE 1. Scoring of Insulin-Like Growth Factor-1 Receptor Expression According to Intensity of Membrane Staining

Score	Pattern of Immunohistochemical Staining in Invasive Component
0	No staining observed or staining observed in <10% of tumor cells.
1+	A faint or barely perceptible membrane staining in >10% of tumor cells. The cells are only stained in part of their membrane.
2+	A weak to moderate complete membrane staining in >10% of tumor cells.
3+	A strong complete membrane staining in >10% of tumor cells.

stained. Herceptest (DAKO) was used for HER2 assay as described elsewhere, and (2+) and (3+) was defined as overexpression.[9]

The primary antibody for IGF-1R used in this study (clone 24-31) is a mouse monoclonal antibody that is specific for α-subunit of human IGF-1R.[10] Paraffin sections were retrieved in distilled water at 95°C for 40 minutes. Then the sections were incubated with the anti-IGF1R antibody for 30 minutes and were rinsed in EnVision plus (DAKO) for 30 minutes. The reaction product was made visible after incubation in diaminobenzidine for 10 minutes.

Human normal colon mucosa and breast cancer–cultured cellblock was used as positive control. The IGF-1R expression in human colon mucosa was defined as (1+), and we scored (2+), (3+) according to the intensity of the membrane-staining within invasive component in accordance to scoring of HER2 by HercepTest (Table 1, Fig 1) at magnification of ×100 to ×200. When there was heterogeneity in IGF-1R staining within a tumor, the highest score was applied regardless of its area among the tumor.

Statistical Analysis

The results were statistically evaluated by SAS software (version 8.2; SAS Institute Inc, Cary, NC).

Disease-free survival (DFS) was calculated from the date of surgical excision of the primary tumor to the date of recurrence or last follow-up. Prognostic information was masked to the pathologists responsible for evaluation of biologic markers. DFS was calculated for all 210 cases. DFS curves were computed by the Kaplan-Meier method. Correlation between IGF-1R expression and various clinicopathologic factors were analyzed by using Fisher's exact test. Univariate analysis of DFS was performed with the use of log-rank test. *P* values of less than 0.05 were considered to be statistically significant.

RESULTS

Characteristics of the Patients

The median age of study population was 53 years (range, 25-83). The median diameter of invasion was 2.2 cm (range 0.1 to 14.0). The majority of the histologic type was invasive ductal carcinoma. About half of the cases were node negative. The number of cases with Nottingham combined histologic grade 1, 2, and 3 were 12, 37, and 137 cases, respectively.

ER and PR was positive in 154 (73.3%) and 98 (46.7%) tumors. HER2 overexpression was seen in 36 tumors (17.1%; 2+: 2.9%, 3+: 14.2%). See Table 2 for a summary of data on patient characteristics.

IGF-1R Immunohistochemistry

IGF-1R was localized to epithelial compartment including normal breast epithelium, ductal carcinoma in situ, and invasive carcinoma (Fig 1). A weak to moderate (ie, (1+) or (2+)) staining was observed in normal duct epithelium. The majority of invasive carcinoma showed both cytoplasmic and membrane staining. There was heterogeneity of staining inside the same tumor: sporadic or patchy, focal, and diffuse pattern. Heterogeneity of IGF-1R staining was observed in 61 (29%) of 210 cases. Though this intratumoral heterogeneity made scoring difficult in some cases, immunohistochemical staining of IGF-1R was stable and reproducible. The number of cases of IGF-1R score 0, 1+, 2+, 3+ was 24 (11.4%), 94 (44.8%), 25 (11.9%), and 67 (31.9%), respectively.

IGF-1R Expression in Association With Various Clinicopathologic Parameters

There was no correlation between IGF-1R expression and age, size of invasion, presence or absence of axillary lymph node metastasis, and histologic grade. ER, PR, and HER2 status also did not correlate with IGF-1R expression. See Table 3.

Univariate Analysis

The median follow-up period was 5.0 years. The 5-year DFS was significantly better among patients with positive ER expression, and negative HER2 overexpression (Table 4). The patients with invasion less than 2 cm, negative axillary lymph node and positive PR expression had a trend of better prognosis, though it did not reach statistical significance. IGF-1R expression status did not correlate with DFS (Fig 2).

DISCUSSION

We tested the prognostic significance of IGF-1R overexpression on formalin-fixed, paraffin-embedded tissue and found no correlation between IGF-1R expression in primary tumor and 5-year DFS. Because this monoclonal antibody is specific[10] and prognostic value of other known biologic markers was validated within this patient population, we conclude that IGF-1R overexpression has no impact on prognosis of breast cancer in this study. This result is concordant with the Foekins et al[4] report, in which IGF-1R was evaluated in 214 primary breast cancer by ^{125}I-IGF radioimmunoassay (RIA).

Estimates of the proportion of IGF-1R expression that have been derived from previous studies, mostly performed by RIA, vary from 39% to 93%.[5-8] This range of positivity may be due to the sensitivity of RIA, because strong membrane staining of 2+ and 3+ was seen

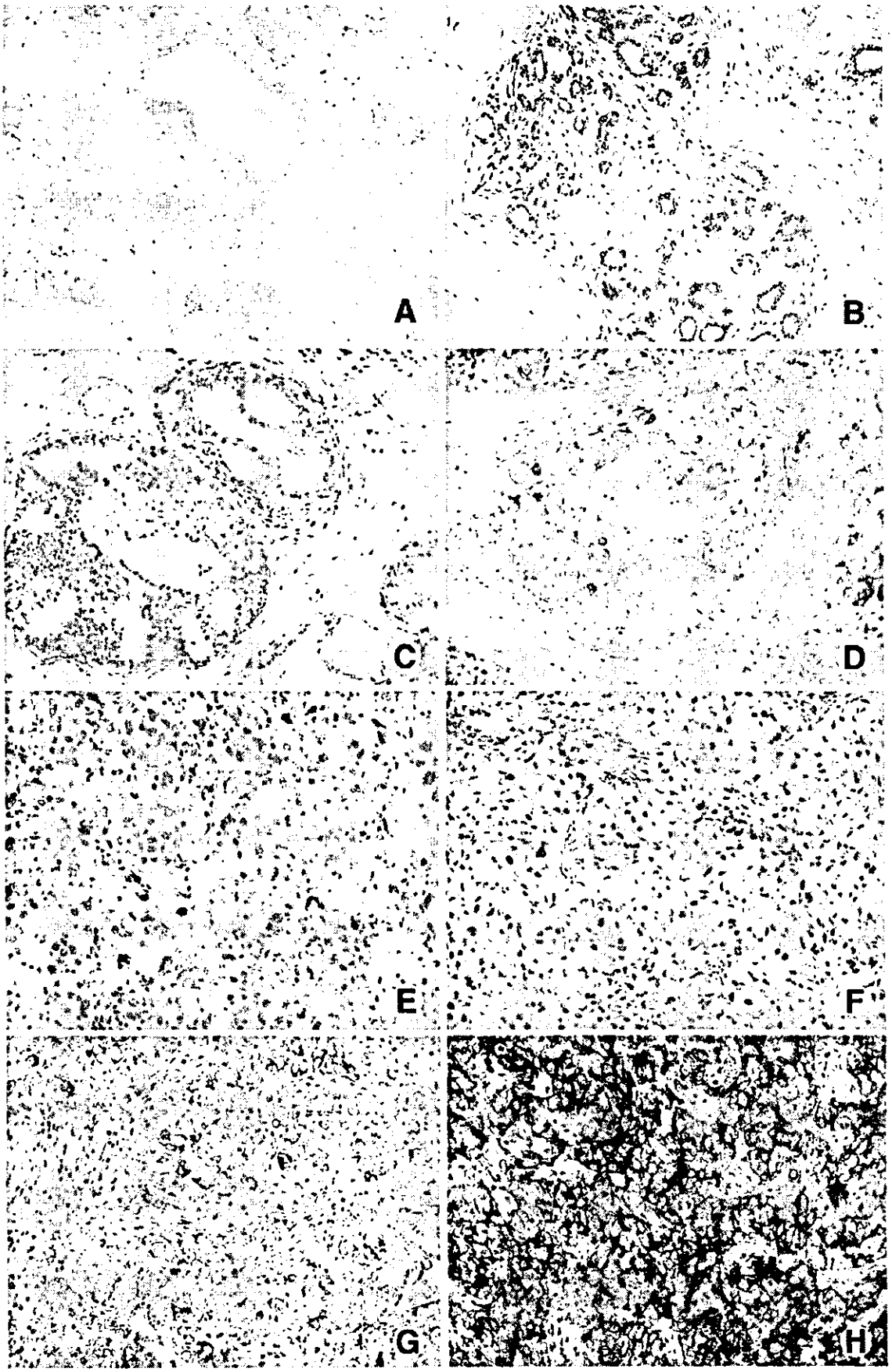

FIGURE 1. Immunohistologic staining of insulin-like growth factor-1 receptor in (A and B) normal epithelium, (C and D) ductal carcinoma in situ, and invasive ductal carcinoma (E-H). IGF-1 receptor expression was scored according to area and intensity of membrane staining (E: score = 0, F: 1+, G: 2+, H: 3+; original magnification, ×100).

TABLE 2. Characteristics of the Patients and Tumors

Parameters	Data
Total	210
Age in yr, range (median)	25-82 (51)
Size of invasion in cm, range (median)	0.1-14.0 (2.2)
Histologic type	
Invasive ductal carcinoma	19
Invasive lobular carcinoma	7
Others	6
Histologic grade	
Grade 1	10
Grade 2	80
Grade 3	120
Axillary lymph node status	
Positive	95
Negative	112
Unknown	3
ER	
Positive	154
Negative	56
PR	
Positive	98
Negative	112
HER2	
0-1	174
2	6
3	30
IGF-1R	
0	24
1	94
2	25
3	67

NOTE. Data are n unless otherwise indicated.

Abbreviations: ER, estrogen receptor, PR, progesterone receptor; HER2, human epidermal growth factor 2; IGF-1R, insulin-like growth factor-1 receptor.

in 43.8%, whereas almost 90% of invasive carcinoma showed moderate staining (scores 1, 2, and 3) in our observation. Happerfield et al[11] reported the localization of IGF-1R staining in benign and malignant fresh-

TABLE 3. Correlation Between Various Factors and IGF-1R IHC score (0/1 vs. 2/3)

Parameters	IHC Score 0/1+	IHC Score 2+/3+	Odds Ratio (95% CI)	Fisher's Exact Test (*P*)
Lymph node status			1.347 (.776-2.337)	.3268
Positive	49	46		
Negative	66	46		
Age (yr)			.932 (.536-1.620)	.8878
<50	51	41		
≥50	67	51		
ER			1.165 (.627-2.165)	.6413
Positive	85	69		
Negative	33	23		
PR			1.174 (.680-2.028)	.5800
Positive	53	45		
Negative	65	47		
HER2			1.032 (.501-2.125)	1.000
0-1	98	76		
2-3	20	16		

Abbreviations: IGF-1R, insulin-like growth factor-1 receptor; IHC, immunohistochemistry; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2.

TABLE 4. Univariate Analysis of DFS by Various Clinicopathologic Parameters

Parameters	5-yr DFS (%)	*P* Values
Lymph node status		0.0670
Positive	68.4	
Negative	79.5	
Age (yr)		
<50	78.3	0.6194
≥50	71.2	
Size of invasion (cm)		0.0667
<2.0	84.3	
≥2.0	66.4	
ER		0.0290
Positive	77.3	
Negative	66.1	
PR		0.1269
Positive	83.7	
Negative	66.1	
HER2		0.0483
0-1	78.4	
2-3	47.2	

Abbreviations: DFS, disease-free survival; ER, estrogen receptor, PR, progesterone receptor; HER2, human epidermal growth factor 2.

frozen tissue by using monoclonal antibody α-IR3 and found high-intensity labeling in all normal mammary epithelium with an intensity that matches that of carcinomas. They observed membrane, cytoplasmic, and mixed staining patterns, which was concordant with our observation. We scored IGF-1R expression according to the intensity of membranous staining, but the role of cytoplasmic IGF-1R has yet to be clarified.

There are several other reports discussing the prognostic value of IGF-1R expression determined by RIA in primary breast cancer. Findings are contradictory: Foekins et al found no relationship between IGF-1R levels,[4] whereas Bonneterre et al[6] and 2 other groups reported IGF-1R as a favorable prognostic factor.[7,8] Because sensitivity of RIA has wide discrepancy as mentioned earlier, further studies by IHC are warranted.

Ouban et al[12] showed the overexpression of IGF-1R by using anti-IGF-1R polyclonal antibody toward the β-subunit of the human IGF-1R in variety of human carcinomas. Bhatavdekar et al[13] suggested that IGF-1R–negative tumor with concomitant hyperprolactinemia might indicate unfavorable prognosis in advanced colorectal cancer. Some data show prevalence of serum or tumor IGF-BP3 within clinical outcome in malignancy, such as breast and prostate cancer.[14,15] In Ewing sarcoma, there was a trend toward increased survival in a high IGF-BP3 to IGF-1 ratio.[16] Because biology of IGF-1R is regulated by a complex endocrine and paracrine system that involves various humoral and local factors, we should take into account those multiple factors that may affect IGF-1R in future studies.

In this study, there was no correlation between IGF-1R expression and ER, PR, or HER2 expression. In previous clinical studies in breast cancer, IGF-1R expression has been reported to have positive correlation with ER expression.[17] However, ER was not necessarily coexpressed in IGF-1R–overexpressed cells in serial sec-

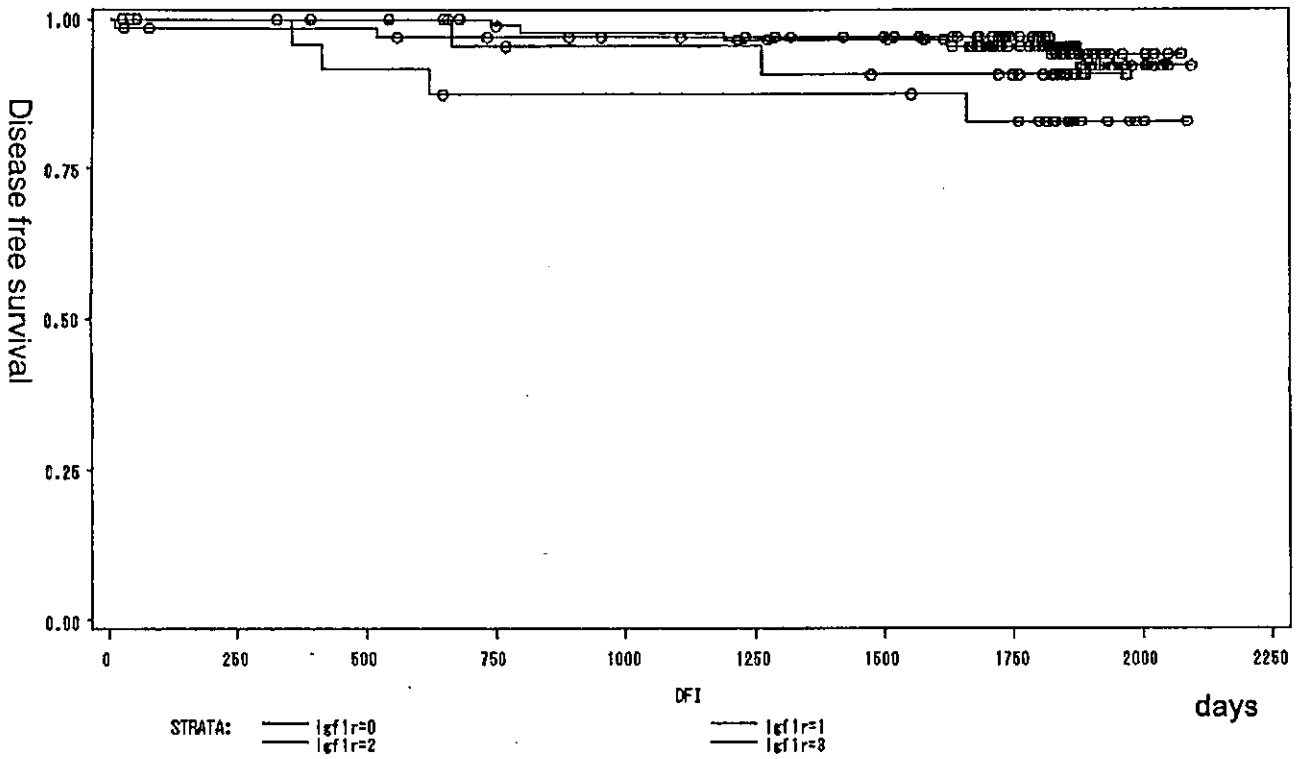

FIGURE 2. Disease-free survival curves for patients group according to insulin-like growth factor-1 receptor expression.

tions in our study. In cellular experiments, there are growing evidences that support reciprocal interaction between estrogens and IGF-1R or between IGF-1 and ER.[18] In terms of HER2, Balana et al[19] suggested existence of hierarchical interaction between IGF-1R and HER2 in regard to HER2 phosphorylation. Multiple signaling pathways are involved in regulation of breast cancer proliferation, apoptosis and metastasis. Technologies such as cDNA array may be useful in understanding the role of IGF pathways in breast cancer.[20]

Though impact of IGF-1R expression on prognosis seems to be limited, IHC is a clinically useful tool for examining protein expression in archive materials. It also resolves the issues of localization and heterogeneity within the tissue. Moreover, blockade of IGF signaling pathway represents an attractive targeted therapy. Preclinical studies of IGF-1R targeted therapy, such as antisense strategies, have shown promising anti-tumor effect, and some are currently under clinical trials.[21-23] Determination of IGF-1R expression by IHC has potential in clinical use in selecting a particular subset of patients that may benefit from IGF-1R targeted therapy.

Acknowledgment. The authors are grateful to Dr. Yuki Kamihara (DAKO Cytomation, Inc) for her contribution in tissue preparation.

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