Neoadjuvant chemotherapy promotes the expression of HER3 in patients with ovarian cancer

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Received June 26, 2020; Accepted September 3, 2020

DOI: 10.3892/ol.2020.12200

Abstract. HER3 (erbB3) signaling serves an important role in the development and chemoresistance of ovarian cancer, and is activated by chemotherapy. To evaluate the influence of neoadjuvant chemotherapy and other clinical factors on the expression of HER3, as well as to examine its role as a prognostic marker, the present study evaluated archived tissues from patients who underwent surgery for ovarian cancer between 2011 and 2018 at our hospital. Immunohistochemical staining for HER3 was performed using formalin-fixed paraffin-embedded surgical specimens and biopsy samples. In total, data from 111 patients with sufficient surgically resected tumor samples were extracted. A total of 28 patients with histology type high-grade serous carcinoma (HGSC) had specimens available from both pre-chemotherapy biopsies and post-chemotherapy surgery. High HER3 expression (HER3-high) was observed in 64 patients (58%), whereas low HER3 expression (HER3-low) was observed in 47 patients (42%). Multivariate logistic regression analysis identified neoadjuvant chemotherapy [odds ratio (OR), 7.49; 95% confidence interval (CI), 2.48-22.64; P<0.001) and non-HGSC histology (OR, 5.42; 95% CI, 1.99-14.78; P<0.001) as significant predictive factors for HER3-high. In pre-chemotherapy biopsy specimens, 15 patients were HER3-high and 13 were HER3-low. After chemotherapy, eight of 13 patients with HER3-low exhibited a change in status to HER3-high, with a trend toward poorer progression-free survival compared to that of patients whose status remained HER3-low. In conclusion, HER3 overexpression was revealed to be common among patients with ovarian cancer, especially in those with non-HGSC histology. In addition, HER3 expression may be promoted by chemotherapy. These findings suggested that patients with ovarian cancer are good candidates for emerging HER3-targeting therapies.

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Key words: HER3 protein expression, ovarian cancer, high-grade serous carcinoma, biomarker, neoadjuvant chemotherapy

Introduction

Ovarian cancer is one of the most refractory and fatal cancers among women worldwide, with approximately 300,000 new cases and 200,000 associated deaths annually (1,2). The majority of patients with advanced disease respond to platinum/taxane therapy; however, most will ultimately experience recurrence and eventually die as a result of disease progression (3,4). Although the biological mechanisms of ovarian cancer invasion, metastasis, and drug resistance remain relatively poorly understood, recent studies revealed HER3 (*erbB3*) signaling plays an important role in development and chemoresistance in ovarian cancer (5,6). The HER3 is a member of the erythroblastic leukemia viral oncogene homolog (ERBB) receptor family, which is often aberrantly expressed and related to poor prognosis in several solid tumors (7-10). HER3 promotes tumor initiation, progression, and treatment resistance mainly through heterodimerization with other ERBB family receptor tyrosine kinases, which activate oncogenic signaling via the PI3K/AKT, MAPK/ERK, and JAK/STAT pathways (11,12). Although HER3 overexpression has been reported to account for 41.3-67.5% of ovarian cancer patients in several studies, it remains unknown what determines whether a patient has high or low HER3 expression (5,13,14). Simpson *et al* (13) suggested early-stage ovarian cancers were more likely than late-stage disease to display intense tumor HER3 staining; however, other studies failed to support the correlation between HER3 expression and disease characteristics, including the International Federation of Gynecology and Obstetrics (FIGO) stage, histologic grade and type, residual disease, age, p53, progesterone and estrogen receptors, EGF receptor, c-MYC, or MDM-2 (5,15-17). However, those studies did not account for the type of previous treatment; therefore, the effect of neoadjuvant chemotherapy on HER3 expression status remains unclear.

Bezler *et al* showed *in vitro* chemotherapeutic drugs such as doxorubicin activated the erbB3/PI3K/AKT signaling in ovarian cancer cells, which could be overcome by blocking the HER2 with trastuzumab (18). We hypothesized that HER3 expression in ovarian cancer patients would be upregulated by chemotherapy and involve chemotherapeutic resistance. In the present study, we investigated how chemotherapy and other clinical factors affect the expression of HER3 in surgically resected ovarian cancer patients and how those factors affect patient prognosis.

Materials and methods

Patients. We retrospectively reviewed medical records of patients who received surgery and were diagnosed with primary ovarian cancer between January 2011 and December 2018 at the National Cancer Center Hospital, Japan. Patients with benign and nonepithelial tumors, borderline tumors, and recurrent disease were excluded from this study. Patients were included in this study if they met the following criteria: i) pathologically diagnosed primary ovarian cancer, ii) with sufficient paraffin blocks of formalin-fixed surgical specimens for evaluation of HER3 immunohistochemistry (IHC). Core needle biopsy specimens obtained before neoadjuvant chemotherapy administration were also retrieved and evaluated for HER3 expression of the representative section which correspond to the histology od the surgically resected specimen if available. Chart reviews were performed and the following information was extracted: Age; performance status at diagnosis; FIGO stage; chemotherapy regimen; dates of surgery, chemotherapy initiation, recurrence confirmation, chemotherapy progression, last follow-up; and survival status. In patients with FIGO stages III or IV, which could not be completely removed, a debulking surgery was performed, if applicable, to reduce the remaining tumor to a diameter not exceeding 2 cm. This study was conducted with the approval of the Ethics Committee of the National Cancer Center Hospital, Japan (2014-393). Written informed consent was obtained from all participants.

Perioperative chemotherapy. Patients diagnosed with clinical FIGO stages III or IV high-grade serous carcinoma (HGSC), where optimal surgery appeared impossible (leaving a residual tumor up to 2 cm in maximal diameter), received neoadjuvant chemotherapy with carboplatin (area under the curve, 6 mg/ml per min) and paclitaxel (180 mg/m^2 on day 1 or 80 mg/m^2 on days 1, 8, and 15) for up to 4 courses prior to surgery. Pathological diagnosis of HGSC was confirmed by needle biopsy or cell block specimens obtained before the induction of neoadjuvant chemotherapy. Patients, except for pathological FIGO stages Ia or Ib with low-grade histology, received postoperative chemotherapy with carboplatin and paclitaxel in line with neoadjuvant chemotherapy within 3 to 4 weeks after primary surgery.

Immunohistochemical staining. For all patients, hematoxylin and eosin-stained slides of surgical and presurgical biopsy specimens obtained before neoadjuvant chemotherapy were reviewed to select representative sections. New 4-μm-thick sections were prepared from paraffin blocks of 10% neutral buffered formalin-fixed surgical specimens and were immunohistochemically stained. Sections were dewaxed, rehydrated, and moistened with phosphate-buffered saline (pH 7.4). After deparaffinization, the expression of HER3 was evaluated by using a rabbit monoclonal antibody against HER3/ErbB3 (1:59 dilution; clone D22C5, Cell Signaling Technology Inc.). Antigen retrieval was achieved by using a PT Link machine (Dako; Agilent Technologies, Inc.) at high pH. IHC staining was performed using the Dako autostainer Link48 (Dako; Agilent Technologies, Inc.) and EnVision Flex Mini Kit (Dako; Agilent Technologies, Inc.), according to the manufacturer's instructions. The slides were counterstained with hematoxylin.

Evaluation of HER3 expression was performed by an experienced gynecological pathologist and two additional observers that independently evaluated HER3 scores in accordance with the HER2 testing guidelines for gastroesophageal cancer from the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology (19). High HER3 expression (HER3-high) was defined as a score of 2+ or 3+, and low HER3 expression (HER3-low) was defined as a score of 0 or 1+; discrepancies were resolved by discussion and consensus. Those pathologist and observers were blinded to clinical data when evaluating the slides.

Statistical analysis. Progression-free survival (PFS) was defined as the time from the date of initial treatment, such as surgery or neoadjuvant chemotherapy, to the detection of any recurrence or death due to any cause. The absence of recurrence was treated as a censored observation.

We used the Mann-Whitney U test to compare continuous variables and the χ^2 or Fisher's exact tests to compare categorical variables between the groups. To identify independent prognostic factors for HER3 expression, a multivariate logistic regression model was applied with forced entry method after adjustment for candidate predictive factors for HER3 expression. PFS was estimated using the Kaplan-Meier method; the corresponding 95% confidence intervals (CIs) were calculated and comparisons between groups were performed by the log-rank test. A statistically significant difference was considered for two-sided $P<0.05$, or $<0.05/n$ (n, number of comparisons) for multiple comparisons according to Bonferroni methodology. All analyses were performed using the Statistical Package for the Social Sciences (SPSS v.26; IBM Corp.).

Results

Patient characteristics. In total, data from 111 patients with sufficient surgical resected tumor samples were extracted from medical records. HER3-high was observed in 64 patients (58%), while HER3-low was observed in 47 patients (42%). Characteristics of patients in each group are summarized in Table I. Most of the patients had FIGO stage I-III disease. Around half of the patients had HGSC histology (56.8%), followed by clear cell carcinoma (26.1%), endometrioid adenocarcinoma (9.0%), and mucinous adenocarcinoma (8.1%). Thirty-four patients (30.6%) received neoadjuvant chemotherapy and subsequent surgery, while 63 patients (56.8%) underwent surgery and thereafter adjuvant chemotherapy. HER3-high was relatively frequently observed in patients with clear cell carcinoma histology (21/29, 72.4%), those with stage IV disease (12/15, 80.0%), or those who had received neoadjuvant chemotherapy (25/34, 73.5%). The

Table I. Patient characteristics at the initiation of treatment.

Characteristics	HER3-high (n=64)	HER3-low (n=47)	P-value
Age, median (range), years	56.5 (36-81)	53 (28-79)	0.140
FIGO stage 2014, n (%)			0.048
I	25 (39.1)	13 (27.7)	
II	6 (9.3)	4 (8.5)	
III	21 (32.8)	27 (57.4)	
IV	12 (18.8)	3 (6.4)	
Histology, n (%)			<0.001
High-grade serous carcinoma	32 (50.0)	31 (66.0)	
Clear cell carcinoma	21 (32.8)	8 (17.0)	
Endometrioid adenocarcinoma	5 (7.8)	5 (10.6)	
Mucinous adenocarcinoma	6 (9.3)	3 (6.4)	
ECOG-PS, n (%)			0.422
0-1	36 (56.3)	30 (63.8)	
2-3	28 (43.8)	17 (36.2)	
Treatment, n (%)			0.069
Surgery alone	8 (12.5)	6 (12.8)	
Surgery plus neoadjuvant chemotherapy	25 (39.1)	9 (19.1)	
Surgery plus adjuvant chemotherapy	31 (48.4)	32 (68.1)	

Mann-Whitney U test was used to compare continuous variables, and χ^2 or Fisher's exact tests were used to compare categorical variables to detect the differences between the groups. A two-sided P<0.05 was considered to indicate statistical significance. ECOG, Eastern Cooperative Oncology Group; FIGO, International Federation of Gynecology and Obstetrics; PS, performance status.

Table II. Multivariate logistic regression of predictive factors for overexpression of HER3.

	Univariate			Multivariate		
Factor	OR	95% CI	P-value	OR	95% CI	P-value
Age ≥55 years (vs. <55)	1.46	0.69-3.11	0.326	1.60	0.70-3.70	0.267
FIGO stage ≥III (vs. ≤II)	0.60	0.28-1.30	0.199	0.46	0.12-1.74	0.255
Non-HGSC histology (vs. HGSC histology)	1.94	0.89-4.21	0.095	5.42	1.99-14.78	<0.001
Neoadjuvant chemotherapy yes (vs. no)	2.71	1.12-6.55	0.027	7.49	2.48-22.64	<0.001

A multivariate logistic regression model was applied to detect the independent prognostic factors for HER3 overexpression, with forced entry method after adjustment for candidate predictive factors for HER3 expression. CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; HGSC, high-grade serous carcinoma; OR, odds ratio.

HER3-high rate was 50.8% for HGSC, 72.4% for clear cell carcinoma, 50.0% for endometrioid adenocarcinoma, and 66.7% for mucinous adenocarcinoma.

Predictive factors for high HER3 expression. We conducted univariate and multivariate logistic analyses to investigate factors associated with HER3-high, and identified neoadjuvant chemotherapy prior to surgery [odds ratio (OR), 7.49; 95% CI, 2.48-22.64; P<0.001] and non-HGSC histology (OR, 5.42; 95% CI, 1.99-14.78; P<0.001) as significant predictive factors for HER3-high (Table II).

Among patients who received neoadjuvant chemotherapy before surgery, pre-chemotherapy biopsy specimens were available in 28 patients who had HGSC histology. The specimens were obtained from the peritoneum (11), omentum (9), ascitic fluid (cell block, 4), and other sites (cervix, vagina, cervical lymph node, and ovary). Changes between pre- and post-chemotherapy HER3 expression status are presented in Fig. 1. In pre-chemotherapy biopsy specimens, 15 and 13 patients showed HER3-high and HER3-low, respectively. After chemotherapy, 8 of 13 patients with HER3-low changed their tumor HER3 expression to HER3-high, resulting in 22 patients in total being

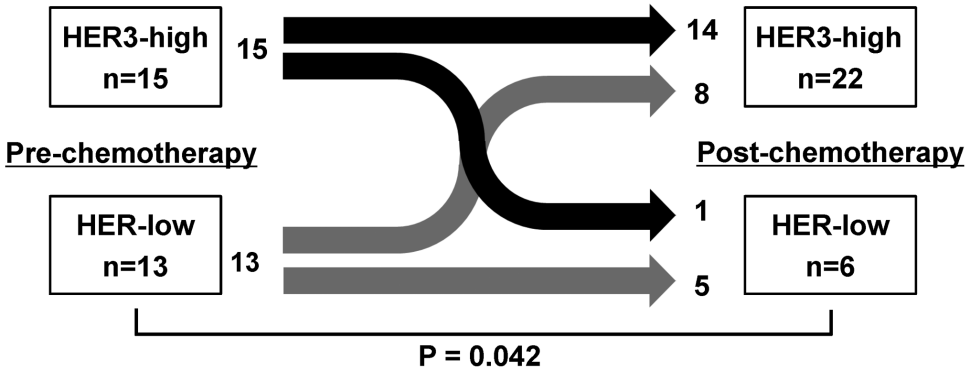

Figure 1. Transition from pre- to post-chemotherapy levels of HER3 expression. The HER3 expression status in biopsy specimens before neoadjuvant chemotherapy (pre-chemotherapy) is shown in the left panel (n=28). The HER3 expression status in surgically resected specimens after chemotherapy are presented in the right panel, showing a significant change from pre- to post-chemotherapy HER3 status (P=0.042, χ^2 test). Eight of 13 patients with baseline HER3-low changed into HER3-high.

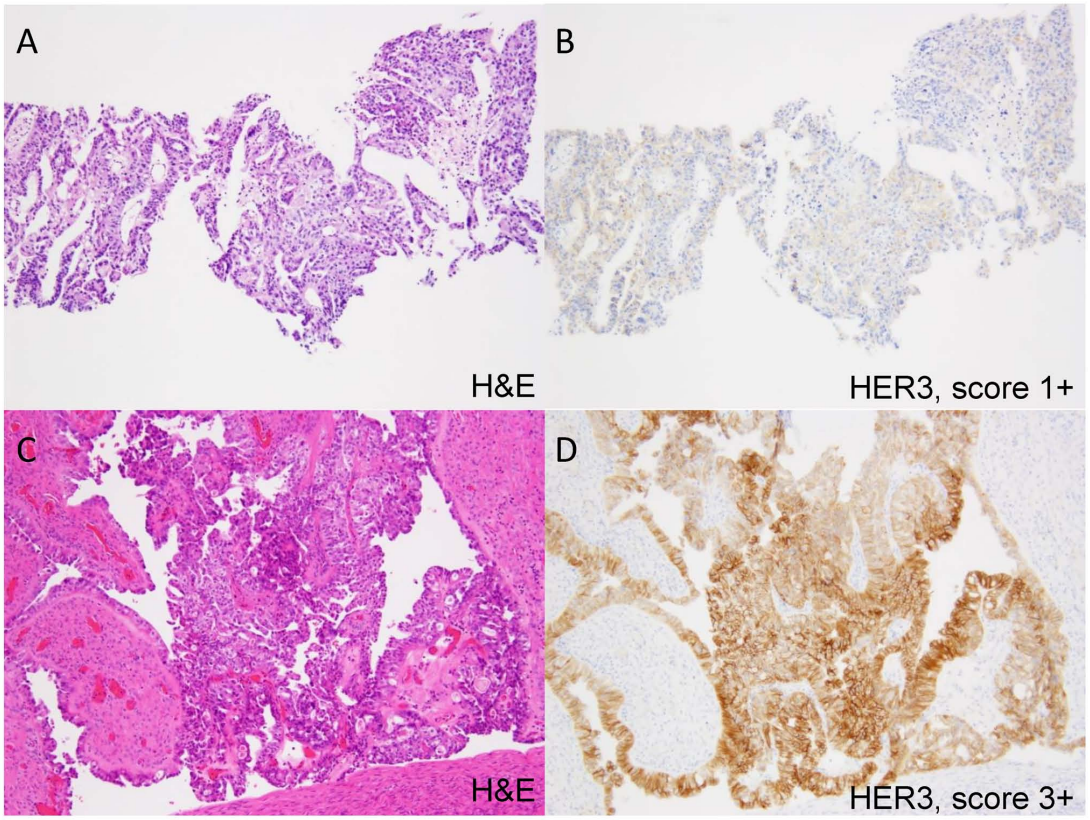

Figure 2. Representative case of upregulated HER3 expression through the course of neoadjuvant chemotherapy. Histological findings and HER3 expression status of a pair of (A and B) pre-chemotherapy biopsy specimens and (C and D) post-chemotherapy interval debulking surgery specimens. (B) Although low-level HER3 expression is detected in a pre-chemotherapy biopsy specimen, (D) HER3 overexpression is observed in high-grade serous carcinoma cells after chemotherapy. Magnification, x200. H&E, hematoxylin and eosin.

classified as HER3-high, with a significant difference from pre-chemotherapy, whereas only 1 of 15 patients changed from HER3-high to -low (P=0.042, χ^2 test). Fig. 2 shows a representative case where HER3 expression was upregulated through the course of neoadjuvant chemotherapy.

Survival analysis. At the point of data cut-off, the median follow-up period was 32.6 months (range, 1.4-89.9 months). To evaluate the impact of HER3 expression on PFS, Kaplan Meier analysis was performed to estimate PFS among HGSC patients. Patients with HER3-high showed a non-significant but consistent trend toward poor PFS (Fig. 3).

In exploratory analysis of patients who received neoadjuvant chemotherapy, patients were divided into four subgroups: Patients who changed their HER3 status from HER3-low to HER3-high (HER3-low-high, n=8) and vice versa (HER3-high-low, n=1); and those who maintained their HER3 status as either HER3-high-high (n=14) or HER3-low-low (n=5). The Kaplan-Meier analysis revealed a trend toward impaired PFS among patients with HER3-high-high and

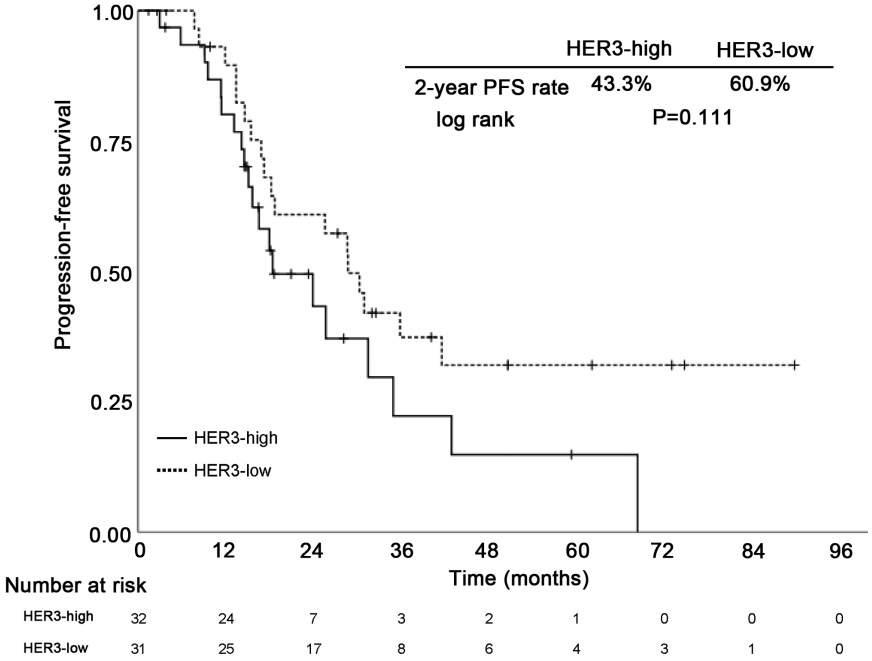

Figure 3. Kaplan-Meier survival analysis of PFS among high-grade serous carcinoma patients stratified by the HER3 expression status. Kaplan-Meier survival analysis of PFS from the date and time of initial treatment, such as surgery or neoadjuvant chemotherapy, to the detection of any recurrence or death due to any cause among high-grade serous carcinoma patients stratified by the HER3 expression status. PFS, progression-free survival.

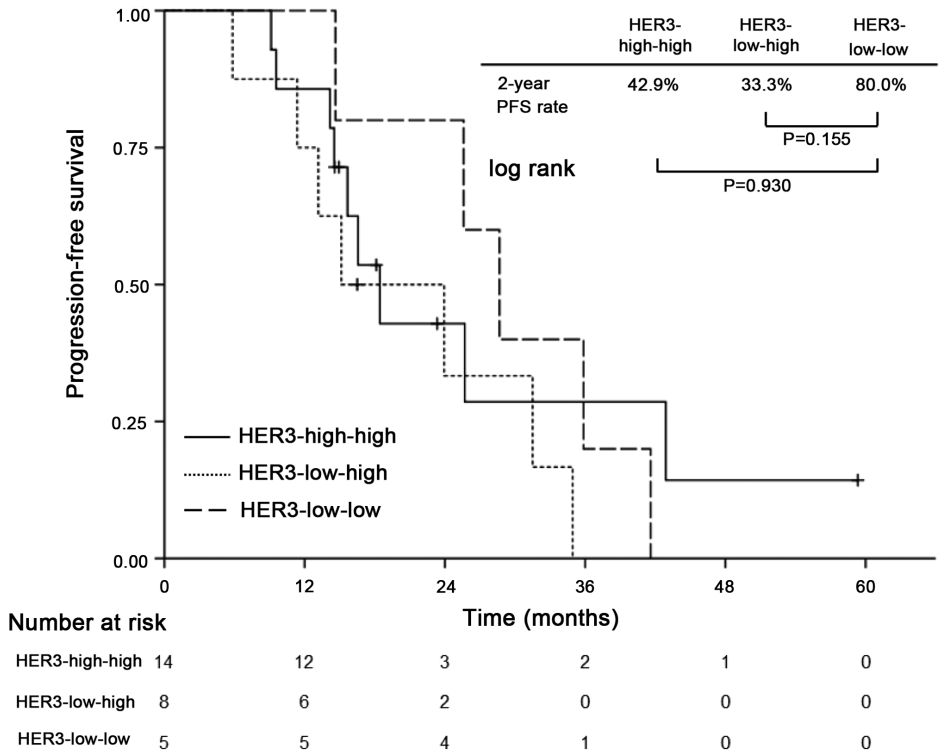

Figure 4. Kaplan-Meier survival analysis of PFS among patients who received neoadjuvant chemotherapy stratified by change to HER3 expression status. Kaplan-Meier survival analysis of PFS from the date and time of initial treatment, such as surgery or neoadjuvant chemotherapy, to the detection of any recurrence or death due to any cause stratified by the transition of HER3 expression status after neoadjuvant chemotherapy. HER3-low-high represents patients who changed their HER3 status from HER3-low to HER3-high through the course of chemotherapy, while HER3-low-low represents patients who maintained their HER3 status as HER3-low. PFS, progression-free survival.

HER3-low-high compared with those with HER3-low-low, but without statistically significant differences (2-year PFS: 42.9, 33.3, 80.0%, respectively; P=0.155 for HER3-low-high with HER3-low-low and P=0.930 for HER3-high-high with HER3-low-low) (Fig. 4). HER3-high-low was omitted because of the small number of cases.

Discussion

This is the first study to demonstrate upregulation of HER3 overexpression through neoadjuvant chemotherapy in surgically resected ovarian cancer patients. In patients with HGSC histology, the frequency of HER3-high was significantly increased between pre- and post-chemotherapy paired samples. HER3-low patients often upregulated their HER3 status to HER3-high with a trend toward impaired PFS, while HER3-high patients rarely attenuated their expression through the course of chemotherapy. Additionally, multivariate analysis revealed that non-HGSC histology and neoadjuvant chemotherapy independently affect overexpression of HER3. In the era of precision medicine, these perspectives would be a step toward a biomarker-based treatment strategy to overcome chemoresistance in ovarian cancer patients.

Over the past two decades, a growing body of evidence has shown that HER3 plays an important role in carcinogenesis, progression, and chemoresistance in solid tumors, including ovarian cancer (7-10,18). Several therapeutic strategies targeting HER3 in human cancers are under development, including monoclonal antibodies blocking ligand binding, promoting HER3 destruction, or locking HER3 in a tethered conformation (20,21). Seribantumab, a fully human immunoglobulin G2 monoclonal antibody targeting HER3 by blocking heregulin (HRG)-mediated HER3 signaling and inducing HER3 downregulation, was evaluated in a randomized phase II study in combination with paclitaxel compared with paclitaxel alone (22). Although the study did not reach its primary endpoint, showing no difference in PFS between two arms, patients with high HRG and low HER2 expression seemed to benefit from the combination of seribantumab with paclitaxel. Recently, manageable safety profiles and antitumor activities of U3-1402, an anti-ERBB3 antibody conjugate with a novel topoisomerase I inhibitor, were reported in two phase I studies in which patients with HER3-positive breast cancer and EGFR mutated non-small cell lung cancer were enrolled (23,24). A phase II/III study (NCT02980341) of U3-1402 targeting HER3-positive breast cancer is ongoing. The high frequency of HER3 overexpression across all histology types (50.0-72.4%) and upregulation of HER3 expression after chemotherapy observed in the current study suggest that ovarian cancer patients are good candidates for HER3-targeting therapies, in particular, patients with clear cell carcinoma, which is often resistant to conventional chemotherapies (25-27).

Our findings raise several questions, including how long the upregulation of HER3 is likely to last and what mechanisms underlie HER3 upregulation. We usually perform surgery 1-2 months after the last administration of neoadjuvant chemotherapy; therefore, HER3 expression seems to be upregulated for at least several months *in vivo*, despite the short half-life of HER3 after activation *in vitro* (28,29) Additionally, Bezler *et al* suggested that metalloprotease ADAM17-mediated long-term upregulation of HER3 ligands in previously treated ovarian cancer cell lines results in activation of HER3 and subsequent AKT phosphorylation (18). Therapeutic strategies that involve combinations of such molecules could be used in targeted therapy for HER3. Concurrently, there is a possibility that HER3-negative clones responding to chemotherapy are expelled by neoadjuvant chemotherapy, and HER3-positive chemoresistant clones are then selected and retained in surgical samples. Further studies are needed to clarify the underlying biological mechanism of HER3 upregulation.

HER3 overexpression has been reported as a factor predictive of worse prognosis in patients with ovarian cancer (5,30). This is expected because HER3 activation is related to one of the mechanisms of chemo-resistance among ovarian cancer patients. We investigated the relationship between HER3-high and PFS among HGSC patients and showed a trend toward worse PFS than that of HER3-low in the present study, although the difference was not significant due to a small sample size. Additionally, patients who changed their HER3 status from HER3-low to -high through the course of neoadjuvant chemotherapy showed a trend toward relatively poorer PFS, as did patients who maintained their HER3-high status. While these findings did not conclusively demonstrate differences between these groups due to small sample sizes, these observations are noteworthy and warrant further exploration.

Our study has several limitations. First, this was a retrospective study conducted at a single institution and included a small number of patients with ovarian cancer. By their nature, such studies are prone to bias, which cannot be adjusted for in multivariate analyses. Second, small biopsy samples acquired from mainly metastatic sites might not reflect the overall primary tumor expression of HER3; therefore, the reported HER3 expression in pre-chemotherapy samples might be an underestimate. In a previous examination of HER2 expression, which compared core needle biopsy and surgical specimens among breast cancer patients, the concordance of HER2 scores between these types of specimens examined by IHC was 90% for 2x2 categories (0-2+ vs. 3+) (31). Although validation of HER3 expression assessment remains required, this previous finding might be applicable to the present study. Third, our study population consisted of relatively few stage IV patients (13.5%), as we only included patients with sufficient surgical specimens. It is uncertain whether our study results generalize to patients with stage IV disease. In a report from the United States, the prevalence of stage IV disease was estimated as 28% of all epithelial ovarian cancer cases. Given the fact that some patients with stage IV disease undergo neoadjuvant chemotherapy and surgery, our results might be relevant to most of ovarian cancer patients. Fourth, the median follow-up period in our study was relatively short. Further examination is needed to evaluate the long-term prognosis in our study population. Moreover, it remains to be elucidated whether the upregulation of HER3 occurs also in platinum-resistant ovarian cancer, whether the upregulated HER3 is also targetable by HER3-targeting compounds such as U3-1402, and whether a sequential or combination strategy is the most efficacious in HER3-targeted therapies for ovarian cancer.

This is the first study to demonstrate that chemotherapy may upregulate HER3 expression in ovarian cancer patients. In this study, non-HGSC histology was a factor predictive of high HER3 expression. In the era of precision medicine, these perspectives contribute toward development of a biomarker-based treatment strategy for this disease.

Acknowledgements

The authors would like to thank Ms. Nao Nakamura and Ms. Kotone Shoji (Department of Breast and Medical

Oncology, National Cancer Center Hospital) for secretarial assistance.

Funding

The National Cancer Center Biobank is supported by the National Cancer Center Research and Development Fund, Japan.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

TM, YK, KY, HY, YS, YoO, HSO, TN, MT, KS, AS, EN, TK, TS, MU, MI, YF, YuO and KT were responsible for the conception and design of the present study, drafted the manuscript, were responsible for the collection and assembly of the data, performed the data analysis and interpretation, and read, revised and approved the final manuscript. All authors read and approved the final manuscript, are in agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the National Cancer Center Hospital (Tokyo, Japan; approval no. 2014-393). Written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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