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CLINICAL TRIAL

Different prognostic significance of Ki-67 change between pre- and post-neoadjuvant chemotherapy in various subtypes of breast cancer

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Abstract In a neoadjuvant setting, three parameters for Ki-67 could be obtained: pre-treatment Ki-67, post-treatment Ki-67 and Ki-67 change between pre- and post-treatments. It is uncertain which of the three parameters has the greatest prognostic significance, and whether this parameter has significance in each subtype of breast cancer. A total of 385 patients who received neoadjuvant anthracycline followed by taxane chemotherapy and subsequent surgery for breast cancer were analyzed retrospectively. By immunohistochemistry (IHC), patients were divided into four subtypes (Luminal A, Luminal B, Triple negative, and HER2). Ki-67 was examined by IHC in pre-treatment core needle samples and post-treatment surgical excision specimens. The relapse-free survival (RFS) rate was compared among each subtype. The median follow-up period was 56 months. The rate of pathological complete response was higher for HER2 (34.8 %) and Triple negative (24.3 %) subtypes than for Luminal B (8.3 %) and Luminal A (3.8 %) subtypes ($p < 0.0001$). A reduction in Ki-67 was observed in 58.5, 83.4, 70.2, and 74.2 % of patients in the Luminal A, Luminal B, Triple negative, and HER2 subtypes, respectively. Ki-67 change between pre- and post-treatments was an independent prognostic factor, but pre-treatment Ki-67 and post-treatment Ki-67 were not independent prognostic factors in a multivariate analysis. The RFS was significantly different between patients whose Ki-67 was reduced and those not reduced for Luminal B (81.4 vs. 50.0 %, $p = 0.006$), Triple negative (74.8 vs. 43.5 %, $p = 0.006$) and HER2 (82.7 vs. 59.0 %, $p = 0.009$). However, for Luminal A, the difference in RFS was not associated with changes of Ki-67 (78.8 vs. 75.3 %, $p = 0.193$). Ki-67 change between pre- and post-neoadjuvant chemotherapy is an independent prognostic factor in patients of Luminal B, Triple negative, and HER2 subtypes. Pre-treatment Ki-67 and post-treatment Ki-67 were not independent prognostic factors in a multivariate analysis.

Keywords Breast cancer · Neoadjuvant chemotherapy · Ki-67 · Prognostic factors · Intrinsic subtype

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Introduction

Neoadjuvant chemotherapy is now well established as a standard treatment option in patients with locally advanced and operable breast cancer [1]. The purpose of neoadjuvant chemotherapy in patients with breast cancer is to not only achieve tumor shrinkage to facilitate the subsequent surgical procedure but also improve clinical outcome by eradicating micrometastases [2–4]. Also, biological and pathological analyses of surgical specimens after neoadjuvant chemotherapy can provide information on predictive and prognostic markers [5–7]. Further progressive research might help to introduce more accurate individualized treatment.

Ki-67 is a nuclear protein with nuclear function that is expressed in all phases of the cell cycle except G0, and it is one of the major markers of tumor proliferation, as assessed by IHC and the Ki-67 antibody, MIB-1 [8, 9].

In addition, Ki-67 has been reported to be an independent predictive and prognostic marker in patients with operable breast cancer [10, 11]. Recently, a meta-analysis concluded that Ki-67 overexpression was associated with worse survival rates both in patients with both lymph node metastasis-positive and in those with metastasis-negative breast cancer [12]. Assessment of Ki-67 is now introduced into daily practice, and used to divide the cancer into subtypes or as a decision-making tool for adjuvant treatment [13].

However, in a neoadjuvant setting, especially neoadjuvant chemotherapy, the role of Ki-67 seems to be somewhat confusing. Physicians can obtain three parameters of Ki-67: pre-treatment Ki-67, post-treatment Ki-67, and Ki-67 change between pre- and post-treatments. It has been reported that a higher pre-treatment value of Ki-67 is associated with a better response to neoadjuvant treatment, and a significant reduction in Ki-67 was observed [14, 15]. On the other hand, previous studies have also reported that Ki-67 assessment performed on samples already exposed to treatment more accurately predicts clinical outcome than does the assessment in pre-treatment biopsy samples [16, 17]. However, this rationale is well established in neoadjuvant hormonal therapy, but not in neoadjuvant chemotherapy. In addition, it is still uncertain which of those three parameters of Ki-67 have the greatest prognostic significance in neoadjuvant chemotherapy settings. To the best of our knowledge, this question has not been investigated until now.

We report here the results of the sequential assessment of Ki-67 in patients who received neoadjuvant anthracycline followed by taxane chemotherapy. Ki-67 was measured both in a pre-treatment biopsy sample and in surgical excision specimens, and then the relationship between three parameters of Ki-67 and clinical outcome was assessed. The primary objective of this investigation is to determine which of the three parameters of Ki-67 have the greatest prognostic significance in a neoadjuvant chemotherapy setting. The second objective of this investigation was to determine whether the most significant parameter of Ki-67 has a prognostic impact in each of the four subtypes characterized by estrogen receptor status (ER), progesterone receptor (PgR), and HER2 status. We hypothesize that the prognostic significance of Ki-67 might not be equivalent in all types of breast cancer but might be depend on subtypes.

Patients and methods

Patients

We retrospectively reviewed the clinical and pathological records of patients who received neoadjuvant anthracycline followed by taxane chemotherapy and subsequent curative surgery at National Cancer Center Hospital East (Kashiwa, Japan) between January 2000 and December 2010. For inclusion in this study, the patients had to be in clinical stage IIA to stage IIIC with histological confirmation, according to the American Joint Committee on Cancer staging (7th edition). All chemotherapy regimens were allowed for this analysis, if the chemotherapy was administered as sequential anthracycline and taxane regimens. Basically, neoadjuvant chemotherapy was administered as four cycles each of anthracycline and taxane-based regimens.

Information on the following parameters had to be available for all patients from the pre-treatment assessment: age, clinical tumor size, clinical axilla nodes status, ER status, PgR status, HER2 status and pre-treatment Ki-67, and also parameters from the post-treatment assessment: chemotherapy agent, axilla nodes status, and post-treatment Ki-67. Patients whose clinical or pathological parameters were not available were excluded from this analysis. Patients who received neoadjuvant hormonal therapy or a combination of chemo-hormonal therapy and who had not undergone surgery were also excluded from this study. Finally, a total of 385 patients were eligible and analyzed for this study. However, only 23 patients (5.9 %) were enrolled from 2000 to 2002, and almost all the patients included in this study were treated between 2003 and 2010. Since most of the promising phase 3 trials with taxane were published after 2000, several standard neoadjuvant chemotherapy regimens existed between 2000 and 2002.

The indication for and type of post-surgical treatment (adjuvant treatment) was based on the St. Gallen Consensus Recommendation at that time. In brief, none of the patients received additional chemotherapy (adjuvant chemotherapy). Patients whose breast cancer was found to be ER and/or PgR positive by immunohistochemistry underwent adjuvant hormonal therapy for at least 5 years. After 2005, if HER2 positivity was confirmed by IHC or fluorescence in situ hybridization, trastuzumab was administered as neoadjuvant or adjuvant treatment for a total of 1 year. In contrast, from 2000 to 2005, none of the patients diagnosed with HER2-positive disease received trastuzumab as neoadjuvant or adjuvant treatment, because trastuzumab was not approved in Japan as adjuvant treatment until 2008 and as neoadjuvant treatment until 2011. All patients who underwent breast-conserving surgery had routinely received adjuvant radiotherapy. In cases of mastectomy, adjuvant radiotherapy was administered only at the discretion of the oncologist.

Immunohistochemistry

Immunohistochemistry (IHC) was routinely performed in our institution using formalin-fixed, paraffin-embedded tissue blocks with both pre-treatment core needle biopsy

samples and post-treatment surgical excision specimens. Immunohistochemical staining of tumors for ER (confirm anti-ER (SP1), rabbit monoclonal antibody, Ventana Medical Systems), PgR (confirm anti-PGR (1E2), rabbit monoclonal antibody, Ventana Medical Systems), and HER2 (pathway anti-HER2 (4B5), rabbit monoclonal antibody, Ventana Medical Systems) were performed using the automated Benchmark XT platform (Ventana Medical Systems) and according to the manufacturer's recommendations. For Ki-67 (Clone MIB1, Dako, Glostrup, Denmark; dilution 1:50), tumors were stained in accordance with the manufacturer's recommendation. All tumor samples and specimens were evaluated by two experienced pathologists belonging to our institution. A cutoff value of $\geq$1 % of positively stained nuclei was used as the definition of ER and PgR-positive disease. HER2 protein positivity was defined as a score of 3 by IHC or as positive by FISH. The methods and procedures of IHC were unchanged through the study period.

Ki-67 expression was quantified using a visual grading system. Cells stained for Ki-67 were counted and expressed as a percentage. If the staining was homogenous, the percentage of Ki-67 positive cells among the total number of carcinoma cells counted was determined at a magnification of 400× using an eye-piece graticule and counting 10 randomly selected fields. When hot spots, defined as areas in which Ki-67 staining was particularly prevalent, were present, pathologists assessed the whole section and recorded the overall average score. Each Immunohistochemical stainings included an external control to validate the Ki-67 protein expression status of each case. Therefore, the same section was used for the external control.

The cutoff level of Ki-67 was defined as $\geq$10 %, because the 10 % Ki-67 cutoff level provided a significant prognostic forecast in our institution in a previous investigation [18]. The categories of Ki-67 change were defined as follows; a reduction group was defined if post-treatment Ki-67 decreased by 1 % or less compared to pre-treatment Ki-67 and a no-reduction group was defined if post-treatment Ki-67 increased by 1 % or more compared to pre-treatment Ki-67 or if the Ki-67 index was unchanged between pre- and post-treatments. The representative figure samples of Ki-67 change with Ki-67 reduction and no-reduction are shown in Figs. 1 and 2.

The subtypes were defined by IHC of core needle biopsy samples as follows according to St. Gallen Consensus 2011 [13]. Luminal A was defined as negative HER2 status, ER positive, and/or PgR positive with Ki-67 $\leq$14 %. Luminal B was defined as negative HER2 status, ER positive, and/or PgR positive with Ki-67 >14 %. The triple negative

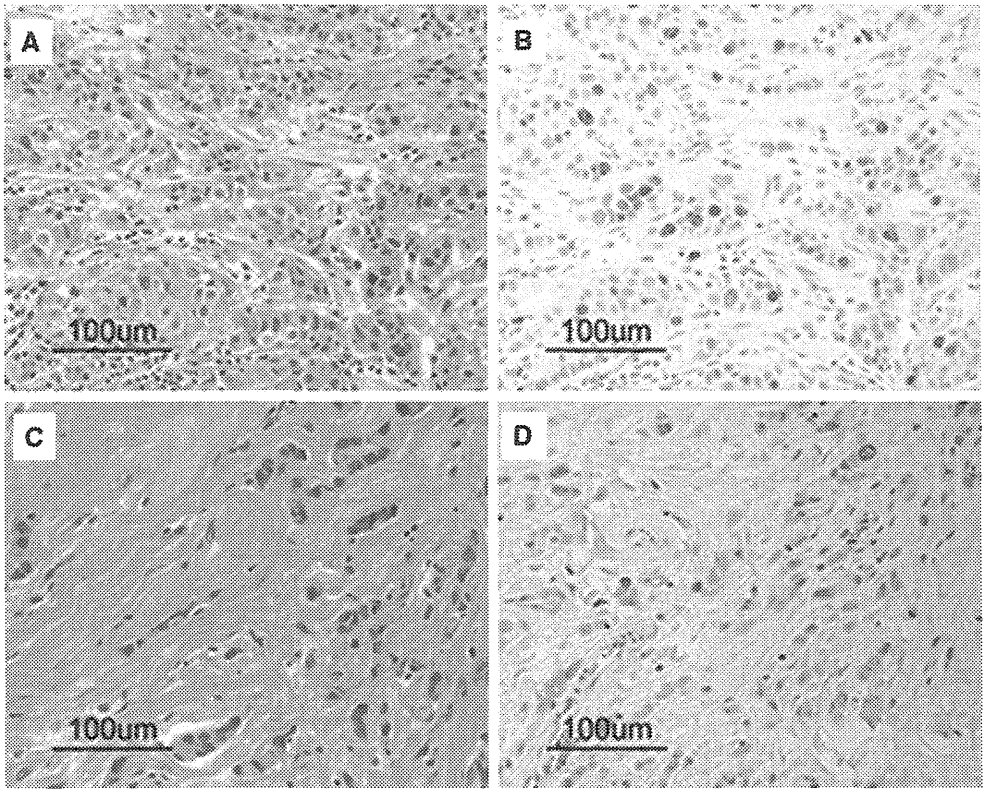

Fig. 1 Representative figure of Ki-67 staining with reduction group. **a** HE of pre-treatment core needle biopsy sample. **b** The Ki-67 score of pre-treatment core needle biopsy would be ~30 %. **c** HE of post-treatment surgical excision specimens. **d** The Ki-67 score of post-treatment surgical excision specimens would be ~4 %

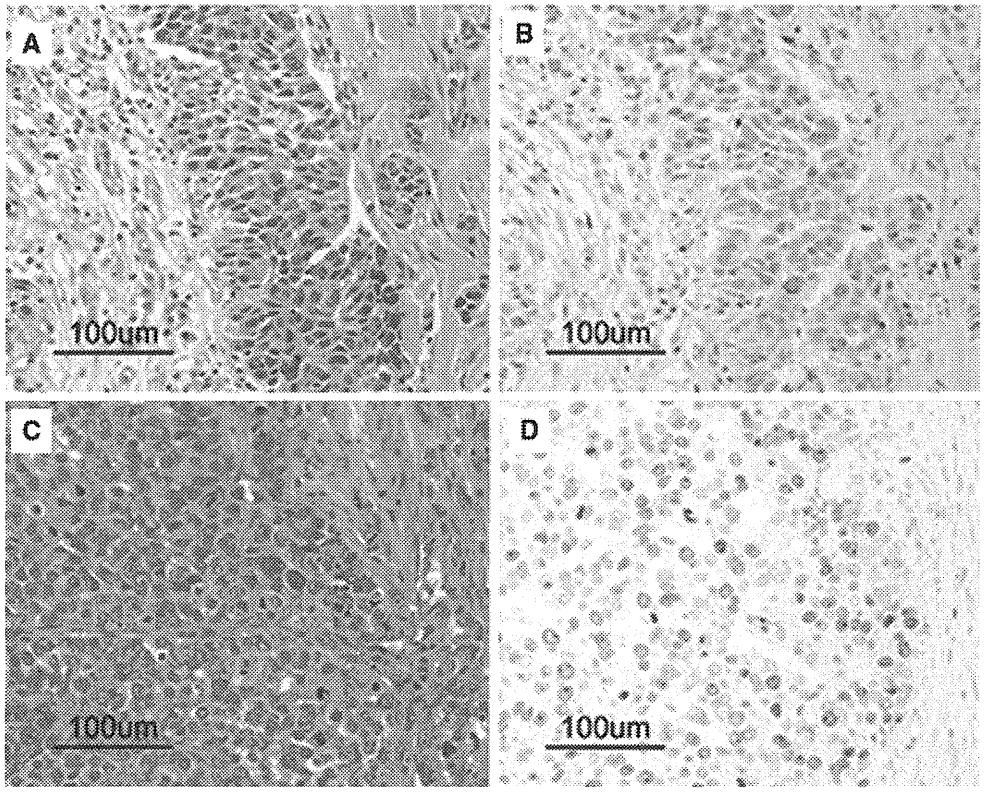

Fig. 2 Representative figure of Ki-67 staning with no-reduction group. **a** HE of pre-treatment core needle biopsy sample. **b** The Ki-67 score of pre-treatment core needle biopsy would be ~20 %. **c** HE of post-treatment surgical excision specimens. **d** The Ki-67 score of post-treatment surgical excision specimens would be ~60 %

subtype was defined as negative HER2 status, ER negative, and PgR negative. The HER2 subtype was defined as positive HER2 status regardless of ER and PgR status.

A pathological complete response (pCR) was defined by the absence of invasive carcinoma in the primary breast tumor irrespective of pathological axillary node status. And then, only the presence of residual ductal carcinoma in situ was included in the pCR category. Post-treatment Ki-67 with pCR was counted as 0 %.

Statistical methods

The definition of relapse excluded local breast relapse, axillary lymph node relapse and newly diagnosed contralateral breast cancer. The relapse-free survival (RFS) period was defined as the interval from the date of surgery to that of the first diagnosis of relapse or the last follow-up date without relapse.

Associations between prognostic factors and RFS were analyzed using Chi-squared test or Fisher's exact test, where appropriate. The Cox proportional hazards model was used for estimation of multivariate analysis. Only prognostic factors that were identified as showing statistical significance in a univariate analysis were included in the Cox proportional hazards regression model. Survival distributions were estimated using the Kaplan–Meier method for RFS, and the Log-rank test was used to compare survival in different strata. All statistical tests were two-sided and had a 95 % confidence interval (CI), with the level of significance established at $p < 0.05$. Statistical analyses were performed using PASW (Predictive Analysis Software) 18.0 for Windows (SPSS, IBM, Chicago, Ill., USA).

Results

Patients and clinical outcomes

Table 1 shows the baseline characteristics of all 385 patients. The median and mean numbers of chemotherapy cycles were 8.0 and 7.06 (range 2–8), respectively. The patients were subdivided into four subtypes by IHC pattern. A total of 106 patients (27.5 %) were classified as Luminal A, 60 (15.5 %) were Luminal B, 107 (27.8 %) were Triple negative, and 112 (29.2 %) were HER2. The details of the distributions of baseline clinical and pathological characteristics among the subtypes are also listed in Table 1. There were significant differences in histological grade (HG) and pre-treatment Ki-67 among the subtype cohorts.

Table 1 Baseline characteristics of 385 patients

Characteristics	Total (%)	Luminal A (%)	Luminal B (%)	Triple negative (%)	HER2 (%)	*p*
No. of patients (%)	385 (100)	106 (27.5)	60 (15.5)	107 (27.8)	112 (29.2)	
Median age (range)	53 (25–71)	51 (25–68)	52 (33–70)	52 (28–68)	53 (32–71)	0.69
Menstrual status						0.74
Premenopausal	171 (44.4)	49 (46.2)	28 (46.6)	51 (47.7)	43 (38.4)	
Postmenopausal	214 (55.6)	57 (53.8)	32 (53.4)	56 (52.3)	69 (61.6)	
Tumor status						0.16
cT1	13 (3.4)	2 (1.9)	5 (8.3)	4 (3.7)	2 (1.8)	
cT2	220 (57.1)	67 (63.2)	30 (50.0)	63 (58.9)	60 (53.6)	
cT3	79 (20.5)	14 (13.2)	15 (25.0)	20 (18.7)	30 (26.8)	
cT4	73 (19.0)	23 (21.7)	19 (16.7)	20 (18.7)	20 (17.9)	
Nodal status						0.084
cN positive	260 (67.5)	61 (57.5)	44 (73.3)	76 (71.0)	79 (70.5)	
cN negative	124 (32.2)	45 (42.5)	16 (26.7)	31 (29.0)	33 (29.5)	
Histological grade						<0.001
1	13 (3.4)	12 (11.3)	0 (0)	1 (0.9)	0 (0)	
2	166 (43.1)	63 (59.4)	38 (63.3)	23 (21.5)	42 (37.5)	
3	130 (33.8)	12 (11.3)	12 (20.0)	57 (53.3)	49 (43.8)	
Missing	76 (19.7)	19 (17.9)	10 (16.7)	26 (24.3)	21 (18.8)	
Median Ki-67 pre-chemo (range)	20.0 (1–80)	9.0 (1–14)	25.0 (15–50)	40.0 (2–80)	20.0 (4–70)	<0.001
Neoadjuvant chemotherapy regimen						0.18
Anthra → PTX	282 (73.2)	77 (72.7)	42 (70.0)	79 (73.8)	84 (75.0)	
Anthra → DTX	103 (26.8)	29 (27.3)	18 (30.0)	28 (26.2)	28 (25.0)	

Follow-up ranged from 9 to 135 months, with a median follow-up of 56 months. During follow-up periods, disease relapse was observed in 105 patients (27.3 %). The RFS at median follow-up of Luminal A, Luminal B, Triple negative, and HER2 subtypes were 77.3, 76.2, 65.4, and 76.5 %, respectively, and the difference in RFS between subtypes was statistically significant (figure not shown; $p = 0.023$).

Response to neoadjuvant chemotherapy in subtypes

The pCR was observed in 74 patients (19.2 %). The rate of pCR was higher for HER2 (34.8 %) and Triple negative (24.3 %) subtypes than for Luminal B (8.3 %) and Luminal A (3.8 %) subtypes ($p < 0.0001$). The reduction in Ki-67 in post-treatment surgical excision specimens compared with pre-treatment biopsy samples was observed in 58.5, 83.4, 70.2, and 74.2 % of patients in the Luminal A, Luminal B, Triple negative, and HER2 subtypes, respectively. The proportion of patients with Ki-67 reduction was significantly different among the four subtypes ($p = 0.003$). The median reduction absolute values of Ki-67 were 5.0, 21.0, 30.0, and 15.5 % in the Luminal A, Luminal B, Triple negative, and HER2 subtypes, respectively. More details on outcome after neoadjuvant chemotherapy are shown in Table 2.

Univariate and multivariate analyses

Various prognostic factors including Ki-67 change, that were commonly validated, were tested for RFS association by univariate analysis. Age, initial tumor size, initial nodal status, ER status, pre-treatment Ki-67, pathological nodal status, post-treatment Ki-67 and Ki-67 change were significant prognostic factors. However, HER2 status and type of taxane were not associated with RFS. More detailed results of the univariate analysis are shown in Table 3.

Prognostic factors that were significantly related to RFS by univariate analysis were then analyzed by multivariate analysis as shown in Table 3. Initial tumor size (Hazard Ratio (HR) 2.37; 95 % CI 1.57–3.45), ER status (HR 2.36; 95 % CI 1.48–3.73), pathological node status (HR 5.84; 95 %CI 3.37–10.12) and Ki-67 change (HR 1.96; 95 % CI 1.17–3.20) were independent unfavorable prognostic factors for RFS. On the other hands, pre-treatment Ki-67 and post-treatment Ki-67 were not independent prognostic factors.

Table 2 Treatment outcomes of neoadjuvant chemotherapy

Characteristics	Total (%)	Luminal A (%)	Luminal B (%)	Triple negative (%)	HER2 (%)	*p*
pCR	74 (19.2)	4 (3.8)	5 (8.3)	26 (24.3)	39 (34.8)	
Median Ki-67, post-chemo (range)	4.0 (0–80)	4.0 (0–50)	4.0 (0–60)	10.0 (0–80)	4.5 (0–70)	
Ki-67 change						
Decreasing	269 (69.8)	61 (57.5)	50 (83.4)	75 (70.2)	83 (74.1)	
Increasing	70 (18.2)	19 (18.0)	7 (11.7)	22 (20.5)	22 (19.6)	
No change	46 (12.0)	26 (24.5)	3 (5)	10 (9.3)	7 (6.3)	

Table 3 Univariate and multivariate analyses for factors related with RFS

Variables	Univariate			Multivariate		
	HR	95 %CI	*P* value	HR	95 %CI	*P* value
Age						
≥35 years	1			1		
<35 years	2.82	1.09–7.32	0.027	1.42	0.70–2.88	0.329
cT status						
≤T2	1			1		
>T2	3.04	1.92–4.83	<0.001	2.37	1.57–3.45	<0.001
cN status						
Negative	1			1		
Positive	2.98	1.70–5.24	<0.001	1.28	0.74–2.27	0.378
Pre-therapy ER						
Positive	1			1		
Negative	1.79	1.14–2.83	0.011	2.36	1.48–3.73	<0.001
Pre-therapy HER2						
Positive	1					
Negative	1.04	0.63–1.70	0.89	NS		
Ki-67 pre-chemo						
<10 %	1			1		
≥10 %	2.11	1.19–3.72	0.009	1.92	0.99–3.36	0.052
Taxane						
Paclitaxel	1					
Docetaxel	0.91	0.53–1.58	0.784	NS		
pN status						
Negative	1			1		
Positive	6.67	3.87–11.48	<0.001	5.84	3.37–10.12	<0.001
Ki-67 post-chemo						
<10 %	1			1		
≥10 %	3.94	2.46–6.31	<0.001	1.26	0.70–2.17	0.470
Ki-67 change						
Reduction	1			1		
No-reduction	2.67	1.68–4.23	<0.001	1.96	1.17–3.20	0.010

The prognostic association of Ki-67 change in each subtype

Figure 3 shows the RFS curves according to Ki-67 change (Ki-67 reduction or Ki-67 no-reduction) in all patients. The RFS rate is 91.2 % with Ki-67 reduction, and 59.4 % with Ki-67 no-reduction. The difference in RFS between two groups was statistically significant (Log-rank, $p < 0.0001$).

We divided patients into four subtypes in accordance with IHC patterns (Luminal A, Luminal B, Triple negative, and HER2). In addition, they were re-classified according to Ki-67 change, and RFS was calculated. In the Luminal A

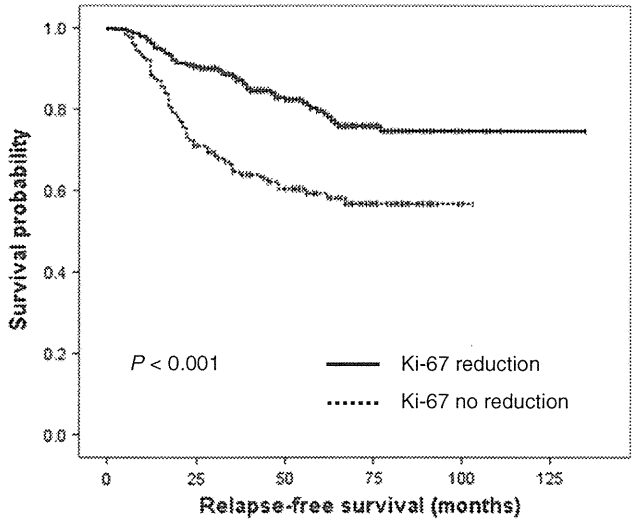

Fig. 3 Kaplan-Meir curves of relapse-free survival by Ki-67 change in all patients

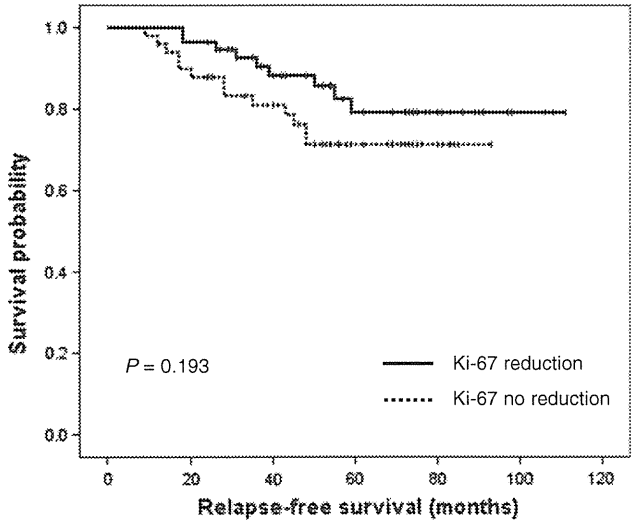

Fig. 4 Kaplan-Meir curves of relapse-free survival divided from Ki-67 change in patients with Luminal A

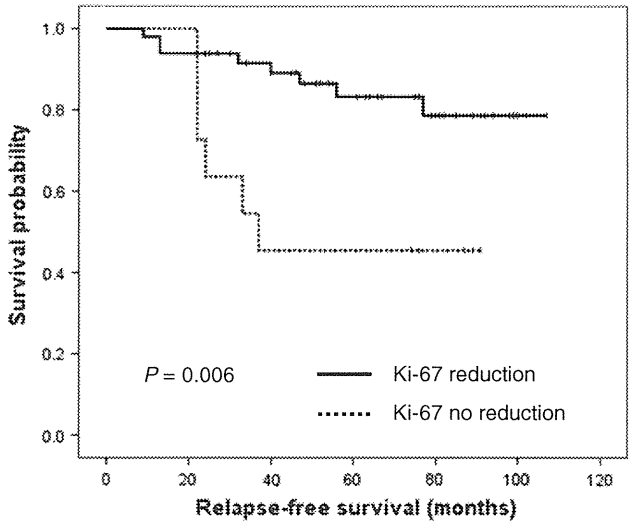

Fig. 5 Kaplan-Meir curves of relapse-free survival divided from Ki-67 change in patients with Luminal B

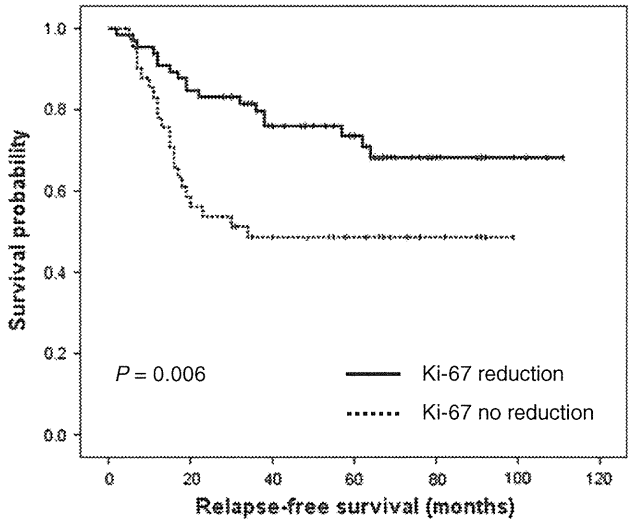

Fig. 6 Kaplan-Meir curves of relapse-free survival divided from Ki-67 change in patients with Triple negative

subtype, no statistically significant RFS difference was observed between Ki-67 reduction and Ki-67 no-reduction in RFS (82.5 vs. 71.3 %, $p = 0.193$, Fig. 4). In contrast, the differences in RFS between Ki-67 reduction and Ki-67 no-reduction patients were statistically significant for Luminal B (83.3 % vs. 45.5 %, $p = 0.006$, Fig. 5), Triple negative (76.0 vs. 48.5 %, $p = 0.006$, Fig. 6), and HER2 subtype (83.0 vs. 60.9 %, $p = 0.009$, Fig. 7) subtypes.

Discussion

Many previous studies have investigated a potential relationship between Ki-67 and survival outcome in early stage breast cancer [10, 11, 19]. However, the Ki-67 assessment seems to provide conflicting results in neoadjuvant chemotherapy settings, mainly because after surgery, three parameters of Ki-67 can be obtained, such as pre-treatment Ki-67 in core needle biopsy samples, post-treatment Ki-67 in surgical specimens and Ki-67 changes between pre- and post-treatment comparisons. It has been uncertain which of the Ki-67 parameters has the greatest prognostic significance. Recent progress might reveal biomarkers that depend on cancer cell biology, such as intrinsic subtypes in breast cancer. We theorized that the prognostic significance of Ki-67 parameters might not be equivalent but depend on subtypes, so therefore we investigated the Ki-67 prognostic

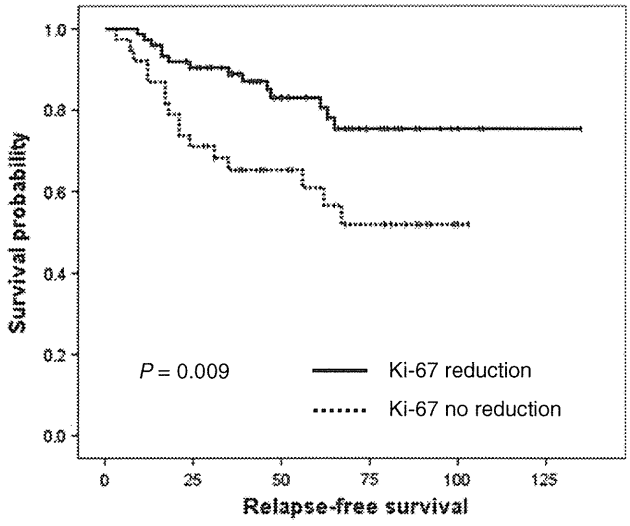

Fig. 7 Kaplan-Meir curves of relapse-free survival divided from Ki-67 change in patients with HER2

association with the subtypes defined by ER, PgR, and HER2 status.

In the present investigation, the three Ki-67 parameters, such as pre-treatment Ki-67, post-treatment Ki-67 and Ki-67 change were significant prognostic factors in univariate analysis. However, in a multivariate analysis, Ki-67 change was the only independent prognostic factor. This result could be interpreted as showing that Ki-67 change is the most important predictor for clinical outcome rather than both absolute values of pre- and post-treatment Ki-67. However, some previous studies reported different results from ours. Lee et al. [20] reported that post-treatment Ki-67 was the only significant independent factor associated with overall survival. In contrast, other studies have not found an independent association between post-treatment Ki-67 and survival [21–23]. In addition, other studies have found the same results as ours that Ki-67 change was a significant independent predictor for disease-free survival (DFS) and RFS [24, 25]. There are some plausible reasons for the discordant results, such as patient population, chemotherapy regimens, and heterogeneous patient subtypes. Thus, the patient proportions of subtypes defined by ER, PgR, and HER2 status might have influenced the results. For example, 43.6 and 52.4 % of patients had ER positive status in the previous study and in our study, respectively, in which post-treatment Ki-67 was found to be an independent prognostic factor [20]. Further investigations assessing the prognostic significance of Ki-67 in each subtype might prove this hypothesis.

Another finding in the current investigation revealed that the reduction in Ki-67 as a favorable surrogate marker for RFS could be applied to Luminal B, Triple negative, and HER2 subtypes, but not to the Luminal A subtype. To the best of our knowledge, this is the first analysis of the association between prognosis and Ki-67 change in each subtype. This finding also means that the significance of Ki-67 change differs among subtypes, and this difference depends on the breast cancer cell biology. Similar findings have been reported in a recently published pooled analysis that investigated the association between pCR and survival in each intrinsic subtype [26]. This pooled analysis, in which 6,377 patients with breast cancer received neoadjuvant anthracycline-taxane-based chemotherapy, demonstrated that pCR is a suitable surrogate marker for patients with Luminal B/HER2-negative, Triple negative, and HER2 subtypes but not for those with Luminal B/HER2-positive or Luminal A subtype. Both our results and this pooled analysis suggest that in low proliferating breast cancer, such as Luminal A, pathological outcomes after neoadjuvant chemotherapy, Ki-67 changes or pCR, are not surrogate markers for prognosis. In contrast, in high proliferating breast cancer, such as Luminal B, Triple negative, and HER2, those parameters could discriminate accurately between patients with good and poor prognosis.

In addition, in a neoadjuvant hormonal therapy setting, opposite results from ours were reported. A previous study on ER-positive breast cancer demonstrated that higher Ki-67 associated with worse RFS following just two weeks of neoadjuvant hormonal therapy compared to the baseline pre-treatment Ki-67 [17]. However, in our investigation, Ki-67 change was not independent prognostic factor for RFS in Luminal A subtype patients treated with neoadjuvant chemotherapy. From those results, the role of Ki-67 change might depend on not only subtype but also treatment procedure (chemotherapy or hormonal therapy).

Gene profiling assays, such as MammaPrint or Oncotype DX, also seem to be useful tools for predicting disease recurrence, and have already been introduced commercially in predictive tests. However these assays are unlikely to become widely used in daily practice at the present time due to issues of cost and insurance coverage. Moreover, to the best of our knowledge, few investigations have confirmed the utility of these gene profiling assays in neoadjuvant settings.

The strengths of our analysis are large sample size, long median follow-up period and treatment of all patients with anthracycline followed by taxane chemotherapy in a single institution. On the other hand, some study limitations are present, such as retrospective analysis, the lack of central pathological review of Ki-67 measurements, and not all HER2-positive patients received trastuzumab as neoadjuvant and/or adjuvant treatment. This treatment difference with or without trastuzumab might influence Ki-67 changes and PFS.

In conclusion, our investigation revealed that in Luminal B, Triple negative, and HER2 subtypes, a Ki-67 change

between pre- and post-treatments is an independent prognostic factor in patients receiving neoadjuvant anthracycline followed by taxane chemotherapy. Pre-treatment Ki-67 and post-treatment Ki-67 were not independent prognostic factors in this patient population.

Acknowledgments The study was carried out in accordance with the Declaration of Helsinki and Japanese ethical guidelines for epidemiological research.

Conflict of interest The authors declare that they have no conflict of interest.

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Case Report

Ectopic Cervical Thymoma: A Case Report with ^{18}F-fluorodeoxyglucose Positron Emission Tomography Findings

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Ectopic thymoma is considered to arise from ectopic thymus tissue deposited as a result of the abnormal mislocalization of thymus tissue during the embryonic stage. An 86-year-old man visited our hospital with chief complaints of hoarseness and a mass in his anterior neck. A preoperative needle biopsy of the mass did not yield a definitive diagnosis. A positron emission tomography (PET) study revealed heterogeneous accumulation of ^{18}F-fluorodeoxyglucose (FDG) in the tumor. The tumor, affecting the left sternocleidomastoid muscle, the recurrent laryngeal nerve, the internal carotid vein, and the brachiocephalic vein, was resected using a combination of a collar incision in the neck and a median incision in the sternum. Immunohistochemically, the tumor was diagnosed as an ectopic thymoma of the neck. To date, only a few cases of ectopic thymoma presenting with FDG accumulation have been reported. Our experience indicates that ectopic thymoma should be kept in mind during the differential diagnosis of neck tumors with FDG accumulation appearing on PET images.

Key words: ectopic thymoma, thyroid tumor, positron emission tomography (PET)

The thymus develops on the ventral side of the third and fourth branchial arch, and descends to the anterior mediastinum during intrauterine development. Mislocalization of thymus tissue during this process can lead to the development of ectopic thymus gland tissue at various sites [1]. Ectopic thymoma developing from such ectopic thymic tissue is a very rare disease. We recently encountered a case of ectopic type A thymoma developing from the thyroid gland; this lesion was difficult to distinguish from a thyroid tumor preoperatively. Similar to intrathoracic thymomas, the ectopic thymoma in the neck region in this case also showed enhanced accumulation of ^{18}F-fluorodeoxyglucose (FDG) on positron emission tomography (PET), making preoperative diagnosis difficult.

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Case Report

The patient was an 86-year-old man. He visited our hospital with chief complaints of hoarseness and an elastic-soft mass in the anterior neck that had increased in size rather rapidly. A hematological examination revealed evidence of renal dysfunction (creatinine level, 1.61 mg/dl) and an elevated thyroglobulin level (112 ng/ml), but no thyroid function

abnormality or abnormal elevation of the serum carcinoembryonic antigen (CEA) level. A detailed examination using a laryngoscope revealed left recurrent laryngeal nerve paralysis. A neck CT revealed a low-attenuation mass (75 × 64 mm) in the left lobe of the thyroid that was not associated with any cervical lymph node enlargement (Fig. 1). Magnetic resonance imaging (MRI) of the neck revealed a tumor that was iso-intense with muscle on T1-weighted images (Fig. 2A), while a fat-suppression (short TI inversion recovery) T2-weighted image revealed the fibrous septum as a low-intensity area in the tumor (Fig. 2B). Tumor infiltration of the left sternocleidomastoid muscle and the left internal carotid vein was suspected, whereas tumor infiltration of the left brachiocephalic vein, trachea or esophagus could not be ascertained during the MRI examination. Because of the patient's impaired renal function, contrast material was not used for the CT or MRI examinations. PET/CT examinations revealed heterogeneous FDG accumulation in the tumor, with a maximum standardized uptake value (SUV-max) of 7.77 (Fig. 3). No abnormal FDG accumulation was noted at any site other than in the tumor. In view of the clinical course (relatively rapid increase in size) and the findings obtained using diagnostic imaging, the patient was suspected of having a malignant tumor (*e.g.*, undifferentiated thyroid cancer). A core needle biopsy (CNB) of the tumor revealed a dense proliferation of cells with relatively

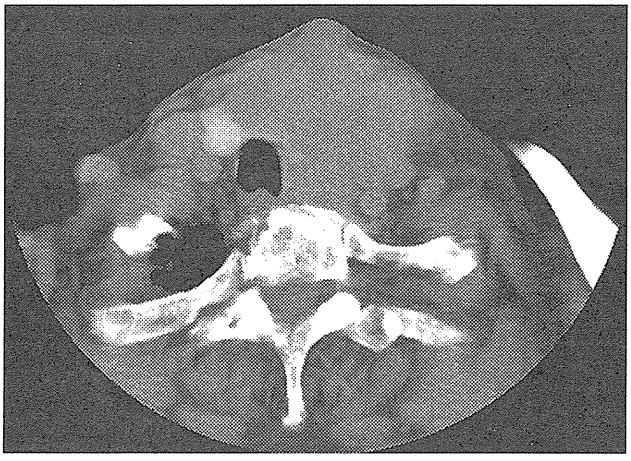

Fig. 1 CT image shows a low-attenuation mass (75 × 64 mm) in the left lobe of the thyroid.

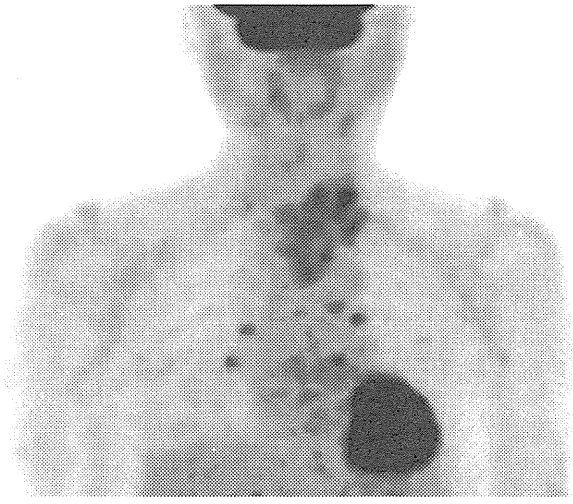

Fig. 3 PET/CT image shows the heterogeneous accumulation of FDG in the tumor (SUV-max: 7.77).

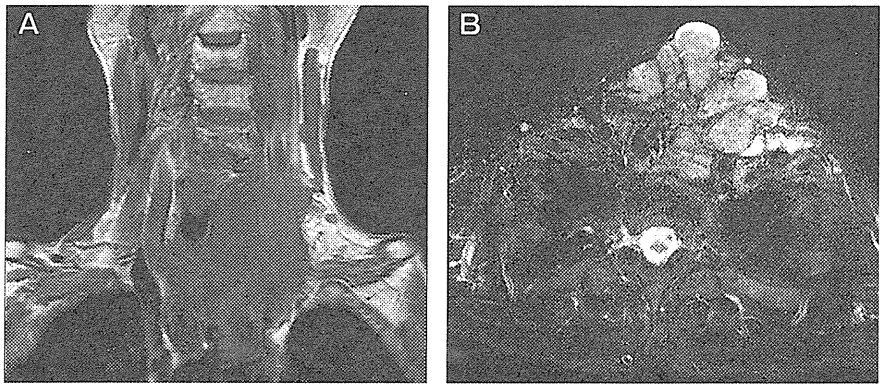

Fig. 2 (A) T1-weighted MRI image shows a tumor iso-intense with the muscle in the anterior neck; tumor infiltration of the left sternocleidomastoid muscle and left internal carotid vein was suspected. (B) A fat-suppression (short TI inversion recovery) T2-weighted image reveals the fibrous septum as a low-intensity area in the tumor.

uniform short spindle-shaped nuclei. Immunohistochemically, the tumor was positive for cytokeratin AE1 and AE3, and negative for thyroid transcription factor-1 (TTF-1). Thus, an epithelial tumor seemed likely, although a definitive diagnosis could not be established.

Tumor resection was undertaken via a combination of a cervical sleeve incision and a median sternal incision. The tumor lesions, which involved the left lobe of the thyroid, the left sternocleidomastoid muscle, the upper part of the thymus, the left recurrent laryngeal nerve and the left internal carotid vein, were resected together. In the caudal region, the left brachiocephalic vein was also affected by the tumor, suggesting the need for combined resection of the brachiocephalic vein and a prosthetic angioplasty. However, since the patient was of advanced age and had renal dysfunction, the combined resection of the brachiocephalic vein was not performed and the tumor in this area was left unresected. The resected specimen showed an internal lobulated architecture separated by fibrous septae (Fig. 4A). Histopathological examination of the resected specimen revealed that the tumor was mainly composed of poorly atypical quasi-circular/short spindle-shaped epithelial cells (Fig. 4B). Immunohistochemically, the tumor cells were weakly positive for p63, positive for 34betaE12, and negative for CD5 and CEA. Based on these findings, the tumor was diagnosed as an ectopic thymoma, World Health Organization (WHO) tumor type A and Masaoka stage III. Postoperatively, external radiotherapy (60 Gray) was administered for the residual tumor. At present, 12 months after the surgery, the residual tumor shows no sign of apparent re-growth.

Discussion

A thymoma is a tumor that develops from the epithelial cells of the thymus. Such lesions develop mostly in the anterior or superior mediastinum. Ectopic thymoma, developing in the neck, middle mediastinum, or posterior mediastinum, reportedly accounts for about 4% of all thymoma cases [2]. Thymoma of the neck region is considered to develop from ectopic thymus tissue in the neck deposited as a result of abnormalities during the course of the descent of the thymus tissue towards the anterior mediastinum after its intrauterine developments on the ventral side of the third and fourth branchial arch [1]. Although some investigators have reported that ectopic thymus tissue is seen in the neck in 1.8% of all patients with Basedow's disease [3], ectopic thymoma developing from ectopic thymus tissue in the neck is considered to be much rarer. Chan *et al.* analyzed 16 cases of ectopic thymoma of the neck, reporting that thymoma of the neck was more frequent among women than among men (women: men ratio = 7 : 1) and that the mean age of the patients was 42.7 (range: 11–71) years [4]. The present case was an elderly man (86 years) and seems to represent a non-typical case of thymoma of the neck.

In the present case, the preoperative diagnostic imaging findings suggested a malignant tumor, such as an undifferentiated thyroid cancer. CT and MRI

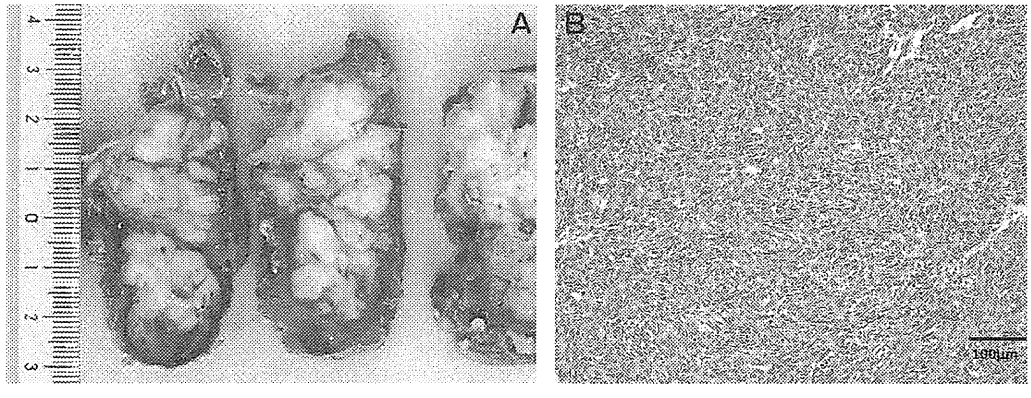

Fig. 4 (A) Resected, formalin-fixed specimen shows an internal lobulated architecture separated by fibrous septae. (B) Histopathological findings show that the tumor was mainly composed of poorly atypical short spindle-shaped cells, leading to a diagnosis of ectopic type A thymoma.

examinations suggested tumor infiltration of the surrounding tissues, including the sternocleidomastoid muscle and the internal carotid vein. However, an adequate evaluation using diagnostic imaging was not possible because contrast material could not be used on account of the patient's impaired renal function. Regarding the diagnostic imaging features of thymoma of the neck, a septal structure visible within the tumor on T2-weighted MRI scans is important [5]. This feature was also seen in the present case and seems to be useful for the diagnosis of this disease. PET/CT examinations revealed heterogeneous FDG accumulation with a high SUV-max value. Even low-risk thymomas are known to be present with enhanced FDG accumulation [6–10]. The SUV-max values of low-risk thymoma (types A, AB, and B1) and high-risk thymoma (types B2 and B3) are significantly lower than that of thymic carcinoma, but no difference is observed between low- and high-risk thymomas [6–8]. FDG accumulation is also usually homogeneous in thymic carcinoma, whereas it is often heterogeneous in thymoma [6]. Although the high levels and homogenous accumulation of FDG in thymic carcinoma can be explained by the high tumor growth rate or cell density, the reason for heterogeneous FDG accumulation in the thymoma is still unclear [6]. In the present case, a correlation between the heterogeneous FDG accumulation and the fibrous septal structure in the tumor was suspected.

Cases of ectopic thymoma of the middle mediastinum presenting with enhanced accumulation of FDG and 11C-acetate have been reported [11]; however, thymoma of the neck with enhanced FDG accumulation has not been previously reported, and the present case may be the first such report. Meanwhile, regarding the accumulation of FDG in thyroid tumors, such accumulations have been reported not only in thyroid cancer, but also in some cases of benign thyroid tumors, such as Hashimoto's disease, subacute thyroiditis and multinodular goiter [12]. Therefore, thymoma of the neck may be difficult to recognize in cases with neck tumors presenting with enhanced FDG accumulation.

In the present case, CNB was performed preoperatively, but a definitive diagnosis was not obtained. We used 18-gauge needles for the tumor biopsy and examined the materials histochemically using immunohistochemical staining. Tumors developing in the thyroid gland usually originate from follicular epithelial cells, C cells and lymphocytes, but some thyroid tumors originate from thymus or parathyroid tissue. Thyroid tumors associated with the thymus include ectopic thymomas, ectopic hamartomatous thymomas, carcinomas showing thymus-like differentiation (CASTLE), and spindle cell tumors with thymus-like differentiation (SETTLE). All of these tumors are very rare [13], and ectopic thymoma represents a diagnostic pitfall especially when using fine-needle-aspiration cytology or frozen sections as specimens, where a lymphomatous process or undifferentiated carcinoma may be suggested [14]. Some investigators have attempted to diagnose cervical thymoma preoperatively using flow cytometry and fine-needle-aspiration biopsy (FNAB) specimens, and this method seems to be useful for the diagnosis of this tumor [15].

In the present case, the tumor was resected via a combination of a collar incision in the neck and a median incision in the sternum. Intraoperatively, the tumor was found to have widely invaded the surrounding organs, affecting the left brachiocephalic vein in the caudal region. Because the patient was elderly and had renal dysfunction, we avoided a combined resection and reconstruction of the left brachiocephalic vein. As a result, the tumor in this region was left unresected, and the operation constituted an incomplete resection. Reportedly, the 5-year survival rate for patients with Masaoka stage III thymoma is significantly lower after incomplete resection (35%) than after complete resection (94%) [16]. The prognosis of ectopic thymoma is unclear because of the small number of reports. Chan *et al.* reported that if an analogy with mediastinal thymomas can be drawn, the circumscribed/encapsulated ectopic thymomas could be curable by surgical resection in almost all cases, whereas the invasive ones may potentially be complicated by recurrence or metastasis [4]. Recently, preoperative induction chemotherapy and chemoradiotherapy for cases of locally advanced thymoma have been reported [17, 18], and further clinical studies evaluating the efficacy of these therapies are needed. Although there may be some room for argument over the validity of adopting such induction therapies for elderly patients with impaired renal function, such as in the present case, these treatments are likely to be essential for younger patients to achieve a complete resection of this tumor using multimodality therapy,

i.e., surgical resection combined with induction therapy or vascular reconstruction.

In conclusion, we encountered a rare case of ectopic cervical thymoma presenting with enhanced FDG accumulation on PET/CT images. Our experience suggests that ectopic thymoma should be kept in mind during the differential diagnosis of neck tumors with FDG accumulation on PET/CT images and a septal structure visible on MRI findings.

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REVIEW

Role of epidermal growth factor receptor in breast cancer

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Abstract Decades of research in molecular oncology have brought about promising new therapies which are designed to target specific molecules which promote tumor growth and survival. The epidermal growth factor receptor (EGFR) is one of the first identified important targets of these novel antitumor agents. Approximately half of cases of triple-negative breast cancer (TNBC) and inflammatory breast cancer (IBC) overexpress EGFR. Thus, EGFR inhibitors for treatment of breast cancer have been evaluated in several studies. However, results so far have been disappointing. One of the reasons for these unexpected results is the lack of biomarkers for predicting which patients are most likely to respond to EGFR inhibitors. Recent studies have shown that EGFR and its downstream pathway regulate epithelial-mesenchymal transition, migration, and tumor invasion and that high EGFR expression is an independent predictor of poor prognosis in IBC. Further, recent studies have shown that targeting EGFR enhances the chemosensitivity of TNBC cells by rewiring apoptotic signaling networks in TNBC. These studies indicate that EGFR-targeted therapy might have a promising role in TNBC and IBC. Further studies of the role of EGFR in TNBC and IBC are needed to better understand the best way to use EGFR-targeted therapy—e.g., as a chemosensitizer or to prevent metastases—to treat these aggressive diseases.

Keywords EGFR · Breast cancer · Targeted therapy · Triple-negative breast cancer · Inflammatory breast cancer

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Introduction

In oncology, the search for new molecular predictors of prognosis (prognostic factors) and response to therapy (predictive factors) is an area of intense investigation. Progress in this area will undoubtedly transform cancer drug therapy from the use of non-targeted antitumor agents in unselected patients to use of targeted antitumor agents in patients selected on the basis of tumor molecular biology. The members of the epidermal growth factor receptor are among the most notable cancer molecular targets identified to date epidermal growth factor receptorepidermal growth factor receptor (EGFR)/ErbB family: EGFR (also known as ErbB1 and HER1), HER2 (also known as HER2/neu and ErbB2), ErbB3 (also known as HER3), and ErbB4 (also known as HER4) [1]. HER2, which is overexpressed in 20–25 % of breast cancers, is the most well-established therapeutic target in breast cancer.

EGFR overexpression in breast cancer is associated with large tumor size, poor differentiation, and poor clinical outcomes [2, 3]. Though EGFR overexpression is observed in all subtypes of breast cancer, EGFR is more frequently overexpressed in triple-negative breast cancer (TNBC) and

inflammatory breast cancer (IBC), which is especially aggressive [4–6]. Treatment of patients with these phenotypes has been challenging not only because of the aggressive behavior of these diseases but also because of the lack of established clinically relevant treatment targets. The role of EGFR in breast cancer has been scrutinized, and several therapies that target EGFR, including gefitinib, cetuximab, lapatinib, and others, have been developed. However, results of clinical studies of EGFR-targeted therapy in breast cancer have been disappointing.

Here, we review the latest studies of EGFR signaling and EGFR-targeted therapies in breast cancer with a special focus on the relationship between EGFR and TNBC and IBC. We summarize the basic biologic characteristics of EGFR and the latest findings from clinical trials of EGFR-targeted therapies for breast cancer.

EGFR in breast cancer

The human EGFR family comprises 4 closely related receptors that are transmembrane glycoproteins containing an extracellular ligand binding domain and an intracellular receptor tyrosine kinase domain. The major signaling pathways activated by EGFR receptors are mediated by PI3 kinase, Ras-Raf-MAPK, JNK, and PLCγ and result in a plethora of biologic functions (Fig. 1) [7, 8]. At the cellular level, the ligands not only induce cell proliferation but also alter adhesion and motility and protect against apoptosis; at the physiologic level, the ligands promote invasion and angiogenesis [9]. Activation of members of the EGFR family promotes scattering and invasion of breast epithelial cells in 3-dimensional culture, which is associated with loss of cell polarization and other features of epithelial differentiation [10]. In vitro, any of these effects may contribute to the malignant phenotype. Dysregulation of EGFR pathways by overexpression or constitutive activation can promote tumor processes including angiogenesis and metastasis and is associated with poor prognosis in many human malignancies [3, 11], [12]. In addition to cross talk between members of the EGFR family, there is evidence for significant interactions between EGFR family members and other receptor tyrosine kinases, such as c-MET and IGF-1R, and it is possible that such alternative signaling pathways are linked to resistance to EGFR-targeted therapies [13].

It has been reported that the expression of both EGFR and HER2 is inversely correlated with estrogen receptor (ER) status, and EGFR–HER2 heterodimers have been shown to increase the metastatic potential of breast cancer cell lines [14]. The rate of overexpression of EGFR is particularly high in TNBC, and the negative impact of EGFR overexpression is particularly pronounced in TNBC. Thus, EGFR has potential as a therapeutic target in TNBC, for which there are no specific targeted therapies at present.

One of the mechanisms of EGFR overexpression is amplification of the *EGFR* gene, which has been described in oligodendroglioma, [15] glioblastoma, lung cancer, [16] gastric cancer, and breast cancer [17]. *EGFR* gene amplification is infrequent in breast cancers overall: previous studies showed EGFR gene amplification in 0.8–14 % of tumors [18, 19]. However, gene amplification has been shown in $\sim$25 % of cases of metaplastic breast cancer, a specific phenotype of TNBC [20–23].

Another mechanism of EGFR overexpression is through activating mutations of *EGFR,* which have been demonstrated in central nervous system tumors and lung cancer, but is rare in breast cancer [24, 25]. Weber et al. [23] found mutations of *EGFR* in 7 of 48 sporadic breast carcinomas and 11 of 24 hereditary breast carcinomas. Surprisingly, mutations were found in both stromal and neoplastic epithelium. These authors also showed that *EGFR* mutations occurred at a significantly higher frequency in hereditary than in sporadic breast cancer ($P = 0.0079$) and that the majority of missense mutations were in the tyrosine kinase domain of EGFR exon 20. These data are in agreement with the fact that the rate of TNBC is higher among patients with hereditary breast cancer than among patients with sporadic breast cancer [26]. Weber et al. suggested that future clinical trials employing molecular targeted therapy should evaluate *EGFR* mutations not only in neoplastic epithelia but also in the surrounding tumor stroma. This will establish the role of *EGFR* mutations in response to therapy and their value in predicting individual variation in response. In breast cancer, as has previously been done in lung cancer (with in-frame deletion of exon 19 and point mutations of exon 21) [27–29], identification of *EGFR* mutations may be used to select patients most likely to respond to EGFR-targeted therapies.

In breast cancer, EGFR expression level or gene mutation status is increasingly being used to select patients for particular treatments. However, whether EGFR is truly a predictive biomarker remains to be proven.

Regulates epithelial-mesenchymal transition (EMT)

In several malignancies, *EGFR* alterations occur at an advanced stage of malignancy characterized by metastatic competence [30–32], and EGFR is thought to promote cancer cell migration and invasion. Recently, EGFR has been shown to promote epithelial-mesenchymal transition (EMT), a process by which cells undergo a morphologic switch from a polarized epithelial phenotype to a mesenchymal fibroblastoid phenotype, in a variety of epithelial cell lines. EMT has been identified as a key process of

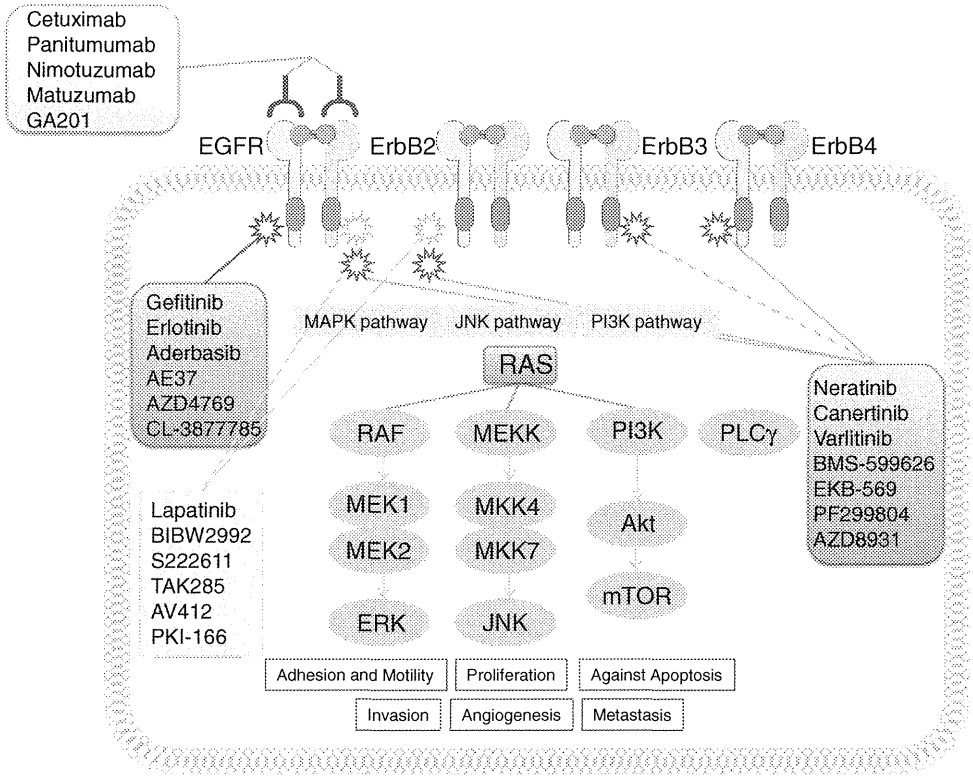

Fig. 1 EGFR inhibitors and downstream signaling pathways. Activation of EGFR leads to homodimerization/heterodimerization and phosphorylation of specific tyrosine residues. The major signaling pathways activated by EGFR receptors are mediated by Ras-Raf-MAPK, JNK, PI3 kinase, and PLCγ and result in a plethora of biologic functions [7, 8]. At the cellular level, the ligands not only induce cell proliferation but also alter adhesion and motility and protect against apoptosis; at the physiologic level, the ligands promote invasion and angiogenesis

migration and tumor invasion [33, 34]. In breast cancer, there is some evidence that EMT is involved in development of the normal mammary gland, but EMT is likely to be most important in tumor progression [35, 36].

EMT is characterized by the loss of epithelial markers (E-cadherin and cytokeratins) and the presence of mesenchymal markers (vimentin and fibronectin). Reduction of the E-cadherin level has been associated with metastatic breast cancer, which indicates the importance of EMT in metastasis [37, 38]. EMT can be induced in vitro in several epithelial cell lines by growth factors such as EGFR, scatter factor/hepatocyte growth factor, fibroblast growth factors, and insulin-like growth factors 1 and 2 [35].

EMT ultimately results in a transcriptional reprogramming of the tumor cell and its transition to a mesenchymal phenotype, promoted by abnormal survival signals through platelet-derived growth factor receptor, fibroblast growth factor receptor, cMET, transforming growth factor beta-receptor, insulin-like growth factor 1 receptor, ERK, and AKT. These proteins and pathways can be targeted by molecular targeted therapies directed toward EGFR, insulin-like growth factor 1 receptor, mammalian target of rapamycin, vascular endothelial growth factor, and cKIT [39]. We have shown that erlotinib, an EGFR-tyrosine kinase inhibitor (TKI), inhibited cell motility and invasiveness and transformed IBC cells from a mesenchymal phenotype to an epithelial phenotype [40]. The fact that cells treated with erlotinib showed higher expression of E-cadherin and lower expression of vimentin suggested that the antimetastatic effect of erlotinib might be through inhibition of EMT [40]. Thus, EGFR is highly involved in EMT and might be a key target for inhibiting tumor metastasis.

Downstream of EGFR, the Ras-ERK pathway has been shown to also regulate EMT, tumor invasion, and metastasis. Activation of RSK by ERK is known to induce mesenchymal motility and invasion in cancer cells [41]. ERK has also been implicated in transforming growth factor-beta signaling: it was shown that transforming growth factor-beta1 induced EMT through activation of ERK1 [42]. However, recently, Ras-induced EMT that produced a dramatic morphological change in nontransformed human epithelial cell lines was shown to involve ERK2, not ERK1. The ERK2-induced EMT involved Fra1, a transcription factor that regulates expression of ZEB1/2, a marker associated with EMT [43].

EGFR in TNBC and basal-like breast cancer

At present, classification of breast cancers on the basis of common molecular features is indispensable for selecting the best treatment strategies. EGFR overexpression is found in at least 50 % of cases of TNBC, which is a higher expression rate than the rates seen in other breast cancer subtypes [44]. Because of the high rate of overexpression

of EGFR in TNBC, EGFR inhibitors are among the targeted agents being developed for treatment of TNBC.

TNBCs are negative for ER, progesterone receptor (PgR), and HER2 and are generally accepted as a clinical surrogate for basal-like breast cancer, one of the intrinsic subtypes based on microarray analysis [45]. EGFR and cytokeratin 5/6 are readily available positive markers for basal-like breast cancer that are applied to standard pathology specimens in clinics. Though TNBC is generally accepted as a clinical surrogate for basal-like breast cancer, it was recently hypothesized that TNBC is heterogeneous, and 50–85 % of TNBC tumors were estimated to be true basal-like breast cancer [46, 47]. Recently, Lehmann et al. [48] reported there are 6 subtypes of TNBC, and the EGF pathway is one of the top canonical pathways for Basal-like 2 and Mesenchymal-like subtypes.

Lee et al. [49] recently showed that EGFR-targeted therapy may be used to enhance the initial sensitivity of TNBC cells to cytotoxic therapy, they identified new strategies to enhance the initial chemosensitivity of TNBC cells. They showed that enhanced cell death observed with the use of time-staggered erlotinib-doxorubicin combinations was directly mediated by sustained EGFR inhibition. After sustained EGFR inhibition, oncogene signatures such as *RAS* and *MYC* signatures were dramatically decreased in TNBC cells. The authors analyzed multiple types of quantitative data by advanced computation network modeling and found that the most effective strategy for killing aggressive TNBC cells was a time- and order-dependent combination of genotoxic agents with small molecule EGFR inhibitors, such as doxorubicin and erlotinib, respectively. They also found that "the enhanced treatment efficacy resulted from dynamic network rewiring of an oncogenic signature maintained by active EGFR signaling to unmask an apoptotic process that involves activation of caspase-8." They concluded that "phosphorylation of EGFR may constitute a useful biomarker of response to time-staggered inhibition in some tumor types that are EGFR driven, such as TNBC and lung cancer."[49].

EGFR in IBC

IBC, the most clinically aggressive subtype of breast cancer, is also associated with EGFR overexpression: 30 % of IBCs express EGFR [50]. Several studies have documented a high frequency of negative ER and PgR status, up to 50 %, and a high incidence of HER2 overexpression, up to 40 %, in IBC tumors [50, 51]. Lack of expression of ER and PgR is clearly one of the reasons for the poor prognosis of IBC, but whether HER2 overexpression has a prognostic role in IBC has yet to be established. In contrast, EGFR overexpression is clearly correlated with poor prognosis in patients with IBC [52]. Patients with EGFR-positive IBC have a worse 5-year overall survival rate than patients with EGFR-negative tumors, and EGFR expression in IBC is associated with an increased risk of recurrence [52].

Because conventional chemotherapy regimens are not sufficient for the treatment of IBC, new therapies for IBC are needed. Among the potential candidates are therapies that target the EGFR pathway. An in vitro study showed that gefitinib, an EGFR-TKI, suppressed the growth of SUM149 cells, which overexpress EGFR and lack ER expression and are widely used as a model of aggressive IBC [53]. Another in vitro study showed that treatment with neutralizing antibody against amphiregulin, one of the ligands of EGFR, decreased EGFR activity, and reduced cell proliferation in SUM149 cells [54]. These findings led to an ongoing study of panitumumab (an anti-EGFR antibody), albumin-bound paclitaxel (Abraxane), and carboplatin in IBC (NCT01036087). This study will elucidate the biologic impact of anti-EGFR therapy on IBC tumors.

EGFR-targeted agents

To date, molecular-targeted agents against EGFR have consisted of small molecule EGFR inhibitors (TKIs) (Table 1) and anti-EGFR monoclonal antibodies (MAbs) (Table 2).

Tyrosine kinases are associated with the cytoplasmic domains of growth factor receptors and oncoproteins, and many tyrosine kinases have the potential to cause transformation if they are mutated or overexpressed. Tyrosine kinases therefore represent an excellent target for the development of cancer drugs [55, 56]. The small molecule inhibitors of EGFR are TKIs which bind to the ATP-binding site in the tyrosine kinase domain of EGFR [57]. TKIs not only act directly on EGFR but also affect the activities of other kinases in the cell; thus, there is some potential for unfavorable side effects. The small molecule inhibitors of EGFR can be classified as pure EGFR TKIs and dual EGFR and HER2 TKIs.

Whereas, small molecule EGFR TKIs are not completely specific for EGFR tyrosine kinase receptors, MAbs are completely specific for the EGFR tyrosine kinase, which could be advantageous. MAbs have less capacity to reach normal intestinal epithelium, which appear to be an advantage for MAb-mediated EGFR blockade since diarrhea was a dose-limiting toxicity with the oral kinase inhibitor such as a Lapatinib, but was not observed with the MAbs in general. Moreover, MAbs could work through other mechanisms, including activation of immune responses through mediating antibody-dependent cell-mediated cytotoxicity that is not seen with the use of small molecule EGFR inhibitors.