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ORIGINAL ARTICLE

Tau expression and efficacy of paclitaxel treatment in metastatic breast cancer

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Abstract
Purpose Paclitaxel is widely used for the treatment of patients with metastatic breast cancer (MBC). Although several mechanisms of paclitaxel resistance have been demonstrated, useful markers of paclitaxel resistance have not been available in clinical practice.
Methods In this study, the clinical significance of tau expression in MBC cases was established by identifying candidates with paclitaxel administration. Tissue specimens obtained from 35 patients were examined. Status of tau expression was determined by immunohistochemistry.
Results Fifteen cases were classified as tau-negative and 20 cases were classified as tau-positive, respectively. Sixty percent of tau-negative expression showed favorable response. Conversely, 85% of tau-positive expression showed progressive or stable disease after paclitaxel administration. Time to disease progression in tau-negative and tau-positive groups was 9.4 ± 6.6 and 6.0 ± 3.7 months, respectively.
Conclusions Patients with tau-positive expression may derive less benefit than tau-negative from paclitaxel therapy in MBC.

Keywords Metastatic breast cancer · Paclitaxel · Tau · Immunohistochemistry

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Introduction

Metastatic breast cancer (MBC) is an incurable disease, and treatment of MBC is palliative in intent. Systemic treatments for MBC usually involve hormone therapy and/or chemotherapy with or without trastuzumab. Anthracycline-based combinations and taxanes can be used for the treatment of MBC as first-line chemotherapy [1–6].

Paclitaxel binds to microtubules and causes suppression of microtubular dynamics, thus inhibiting spindle formation and stopping mitosis in the late G2/M phase. This effect is considered to be the basis of cytotoxic activity by paclitaxel [7]. Various molecules have been suggested as markers for predicting of paclitaxel sensitivity. *p*-Glycoprotein is a pump that, in normal tissues such as the gastrointestinal tract and brain, prevents accumulation of toxic substances [8, 9]. Overexpression of *p*-glycoprotein is thought to be one of the most common mechanisms underlying resistance to paclitaxel in vitro [10, 11]. Although substantial evidence from in vitro systems suggests that *p*-glycoprotein mediates resistance to paclitaxel, demonstrating such roles in vivo has proven to be problematic due to a lack of universally accepted guidelines for analytical or clinical validation, different molecular assay targets (mRNA or protein), and low sensitivity and specificity for currently used methods of IHC [8, 9]. Overexpression of the βIII-tubulin isoform has also been associated with resistance to paclitaxel in a number of human cancer cell lines [12], and the emergence of resistance to paclitaxel in breast cancer cell lines has been correlated to increased abundance of the βIII isoform [13]. Although some clinical studies have failed to link response to paclitaxel with levels of βIII-tubulin expression, others have provided positive results [14]. Phosphorylation of the bcl-2 oncoprotein occurs following treatment with antimitotic drugs through various signaling

pathways, leading to inactivation of bcl-2 and induction of apoptosis [15, 16]. Various in vitro studies have demonstrated that paclitaxel-induced mitotic arrest initiates phosphorylation of bcl-2 on the S70, S87 and T69 serine and threonine amino acid residues [17]. Clinically, breast tumors showing increased expression of phosphorylated bcl-2 show increased sensitivity to paclitaxel compared to tumors with reduced expression [18].

Rouzier et al. recently reported that low expression of microtubule-associated protein tau in breast cancer was associated with higher rates of pathologic complete response (pCR) to preoperative paclitaxel chemotherapy [19]. Tissue arrays from 122 independent but similarly treated patients with breast cancer with preoperative chemotherapy were used for validation by immunohistochemistry (IHC). In total, 74% of pCR cases were tau-negative, and tau-negative status was identified as a significant independent predictor of pCR in paclitaxel sensitivity. Decreased paclitaxel binding and reduced microtubule polymerization in breast cancer cells was also shown after preincubation of tubulin with tau in vitro [19]. In the highlights of the tenth St Gallen international conference on primary therapy for early breast cancer, tau expression was adopted as a candidate of novel marker for paclitaxel response [20].

The purpose of this study was to clarify the significance of tau expression in MBC from the perspective of response to paclitaxel administration. We retrospectively investigated possible correlations between clinical response and tau expression in breast cancer tumor samples from MBC using IHC. Selecting the most appropriate and effective treatment according to the individual biological profile of the tumor is of utmost importance in improving outcomes for patients with MBC. This approach would avoid toxicity in patients defined as resistant to a given chemotherapeutic regimen and would greatly improve the cost-effectiveness ratio by selecting patients sensitive to the same regimen.

Materials and methods

Patients

Subjects comprised 35 eligible patients with MBC in whom clinical response to paclitaxel was evaluable and who had received neither chemotherapy nor radiotherapy before surgery in the Department of General and Gastroenterological Surgery at Osaka Medical College Hospital from 1995 to 2006. Informed consent was obtained from all patients regarding the use of tissue specimens for research purposes. All patients showed an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2. All patients underwent physical examination, chest radiography, and computed tomography of metastatic sites. Bone scan was performed when serum levels of tumor markers were increased, if symptoms were suggestive of skeletal involvement, or if metastatic bone disease had previously been identified.

Chemotherapeutic regimens and evaluations

Paclitaxel was administered at 80 mg/m^2 for 1 h on days 1, 8, and 15, and repeated every 4 weeks. All patients underwent physical examination and assessment of toxicity. Visible lesions were measured before each cycle of therapy. All toxicities were evaluated and scored according to World Health Organization criteria. Radiological assessment of the tumor was repeated every three to four cycles of therapy. Response was evaluated according to RECIST criteria after three to four cycles of paclitaxel. Complete response (CR) was defined as the disappearance of all measurable lesions in $\geq$4 weeks. Partial response (PR) was defined as a >30% decrease in the sum of products of the greatest perpendicular diameters of all index lesions persisting for $\geq$4 weeks without the development of new lesions. Stable disease (SD) was defined as no change in tumor size or change that was too small to qualify as PR or progressive disease (PD), which in turn was defined as an increase of >20% or the appearance of new lesions.

IHC and evaluation of expression status

For immunohistochemical validation, routinely processed formalin-fixed, paraffin-embedded tissue blocks containing the primary breast tumor mass were selected. Serial sections, 4-μm thick, were prepared for tissue slides. Immunostaining was performed by the standard avidin–biotin–peroxidase complex technique using an LSAB kit (Dako A/S, Carpinteria, CA, USA). Before using the LSAB kit, antigen retrieval was achieved by the pressure cooking method, as described previously [21–23]. In brief, deparaffinization and rehydrated sections were bathed in a 10^{-3} M sodium citrate buffer (pH 6.0) after bringing the solution to a boil in a pressure cooker, and then boiled for 20 min while maintaining the pressure. After quenching in 3% hydrogen peroxide and blocking for 5 min, sections were incubated overnight at 4°C with the diluted monoclonal antibody estrogen receptors (1/50, clone 1D5; Dako A/S), progesterone receptors (1/100, clone PgR636; Dako A/S), c-erbB-2 (1/70, clone 3B5; BD Bioscience, San Jose, CA), and tau (1/50, clone 9A1; Immuno-Biological Laboratories, Gunma, Japan), irrespective of phosphorylation status. Biotinylated antimouse immunoglobulin and streptavidin conjugated to horseradish peroxidase were then added. Finally, 3,3′-diaminobenzidin tetrahydrochloride was used for color development, and hematoxylin was used for counterstaining.

Cells were considered positive for estrogen and progesterone receptors only when >10% distinct nuclear staining

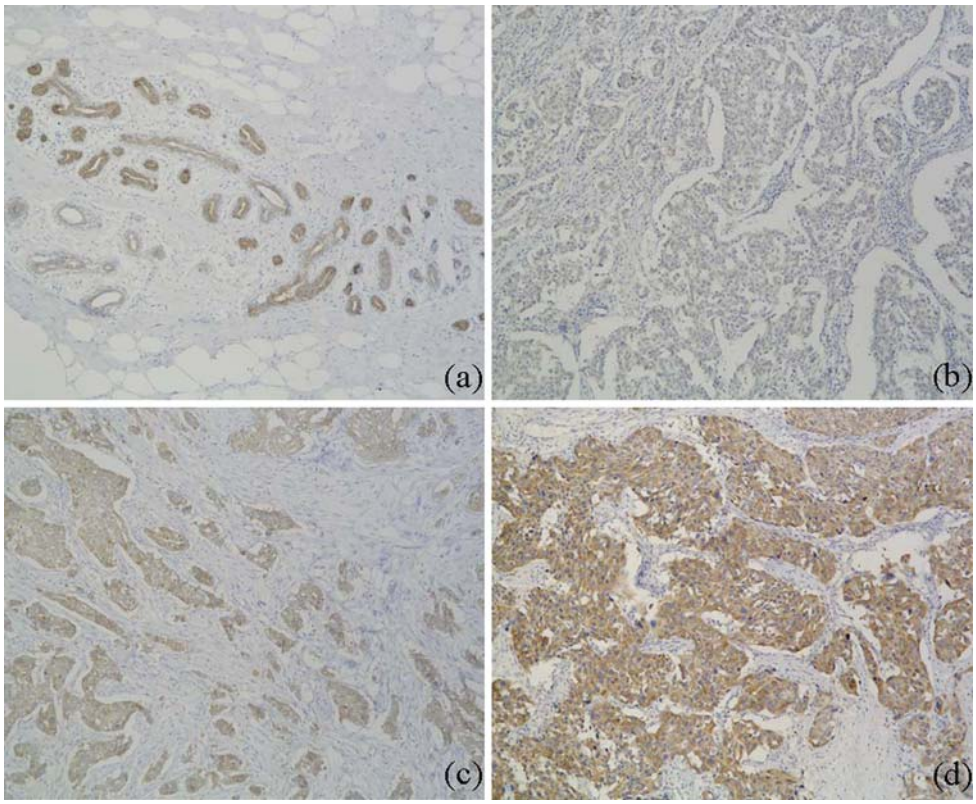

Fig. 1 Tau expression by IHC. **a–d** Tau expression in normal breast epithelium (**a**) and invasive breast cancer with 1+ (**b**), 2+ (**c**) and 3+ (**d**) staining (magnification ×40)

was identified. IHC for c-erbB-2 was scored using only the membrane-staining pattern and intensity of invasive tumor cells. Immunoreactivity was defined as weakly positive (2+) if >10% of tumor cells showed weak-to-moderate complete membrane staining or as intensively positive (3+) if >10% of tumor cells showed strong complete membrane staining. When membrane staining was absent or present in <10% of tumor cells, the reaction was considered negative or 1+, respectively. The scoring method for tau was adopted from that described by Rouzier et al. [19]. Normal breast epithelium served as an internal positive control (Fig. 1a). Omission of the primary antibody served as a negative control. Tau staining of tumor cells was scored as follows: IHC score 0, no staining; 1+, less staining than normal epithelium (Fig. 1b); 2+, similar to normal epithelium (Fig. 1c); 3+, uniform staining more intense than normal cells (Fig. 1d). Cases with 0 or 1+ staining intensity were considered tau-negative, and tumors with 2+ or 3+ staining were considered tau-positive. All staining was evaluated in a blinded manner without knowledge of the clinicopathological parameters or outcomes, and was scored independently by two observers.

Statistical analysis

All statistical analysis was performed using the Excel software for Windows. Differences in clinical factors and molecular markers in paclitaxel response were assessed by using the chi-square test or the Fisher's exact test when necessary because of small individual cell frequencies. Differences in the duration of response and time to disease progression in tau-negative and tau-positive groups were evaluated using the nonparametric Mann–Whitney's *U* test. Continuous variables were summarized as the mean (±standard deviation [SD]) as warranted. Values of $P < 0.05$ were considered statistically significant.

Results

Patient characteristics

Table 1 summarizes main patient characteristics. Median age was 53 years (range 23–73 years). The vast majority of patients (83%) showed good ECOG PS of 0 or 1. Four patients (11%) displayed MBC at diagnosis (Stage IV in UICC TNM classification), and 31 patients (89%) did not. Median disease-free interval after initial breast cancer diagnosis was 29.3 months (range 5–72 months). Thirty-one patients (89%) received four to six cycles of cyclophosphamide, methotrexate, and 5-fluorouracil; three to eight cycles of cyclophosphamide, doxorubicin, and 5-fluorouracil; or six cycles of 5-fluorouracil, epirubicin, and cyclophosphamide as adjuvant chemotherapy. Metastatic sites were visceral in 27 cases (77%), soft tissue in seven cases (20%), and bone in ten cases (29%), respectively. Paclitaxel was

Table 1 Clinical characteristics of 35 patients

	n (%)
Performance status	
0	9 (26%)
1	20 (57%)
2	6 (17%)
Prior adjuvant chemotherapy	31 (89%)
CMF	17 (49%)
CAF	12 (34%)
FEC	2 (6%)
Metastatic sites	
Visceral	27 (77%)
Soft tissue	7 (20%)
Bone	10 (29%)
Number of prior chemotherapies for MBC	
0	28 (80%)
1	7 (20%)
CAF	5 (14%)
CMF	1 (3%)
Capecitabine	1 (3%)

Median age, years (range): 53 (23–73)

CMF cyclophosphamide, methotrexate, and 5-fluorouracil; *CAF* cyclophosphamide, doxorubicin, and 5-fluorouracil; *MBC* metastatic breast cancer

administered as first-line therapy in 20 cases (80%). A total of 277 cycles were delivered, with a median number of six cycles (range 3–22 cycles). Although the first appearance of Grade 3–4 toxicity warranted dose interruption of paclitaxel until recovery, no patients required paclitaxel dose reduction.

Response to paclitaxel

Among the 35 patients evaluable for response, CR was identified in three patients (9%), PR in nine patients (26%), SD in nine patients (26%), and PD in 14 patients (40%). Median duration of response was 8 months (range 4–18 months). Median time to disease progression was 5 months (range 2–21 months), and overall survival was 51.8 months (range 15–130 months).

Evaluation of clinical factors and molecular markers in paclitaxel response

Table 2 presents the evaluation of clinical factors and molecular markers in paclitaxel response in 35 MBC cases. No significant correlation was observed between patient age; lymph node metastasis; line of paclitaxel therapy; expression of hormonal receptor and c-erbB-2; and paclitaxel response. However, a significant difference was identified between paclitaxel effectiveness and tau expression (Table 2). Nine (60%) of 15 cases with tau-negative expression showed favorable response to paclitaxel administration (CR or PR) compared with three (15%) of 20 cases with tau-positive expression. The odds ratio for CR or PR in tau-negative tumors was 8.5 (95% confidence interval 1.7–42.3; $P = 0.01$). In addition, duration of response was 10.7 ± 5.7 months for tau-negative group and 6.0 ± 2.0 months for tau-positive group, and time to disease progression was 9.4 ± 6.6 months for tau-negative group and 6.0 ± 3.7 months for tau-positive group, respectively (Table 3). Although no significant differences were identified, these periods tended to be longer in tau-negative group.

Table 2 Relationship between each variable and response to paclitaxel

	n	Number of responses (%)	*P*
Age (years)			
<50	8	3 (38)	
≥50	27	9 (33)	0.57
LN metastasis			
Negative	8	5 (63)	
Positive	27	7 (26)	0.07
Paclitaxel therapy for MBC (line-of-therapy)			
First	28	9 (32)	
Second	7	3 (43)	0.84
ER			
Negative	22	7 (32)	
Positive	13	5 (38)	0.69
PgR			
Negative	21	7 (33)	
Positive	14	5 (36)	0.88
c-erbB-2			
Negative/1+/2+	29	10 (34)	
3+	6	2 (33)	0.67
c-erbB-2			
Negative/1+	22	9 (41)	
2+/3+	13	3 (23)	0.24
Tau			
Negative	15	9 (60)	
Positive	20	3 (15)	0.01

LN lymph node, *ER* estrogen receptor, *PgR* progesterone receptor

Discussion

The present study focused on IHC of tau expression in primary tumor tissue as a susceptibility marker for paclitaxel therapy against MBC. We anticipate that tau will offer a

Table 3 Duration of response and time to progression for tau-negative and tau-positive groups

Tau	Duration of response (months)	Time to disease progression (months)
Negative	10.7 ± 5.7	9.4 ± 6.6
Positive	6.0 ± 2.0 (NS)	6.0 ± 3.7 (NS)

Values represent mean ± SD

NS nonsignificant

powerful marker for response to paclitaxel, as tau binds strongly to microtubules in the absence of paclitaxel, whereas microtubules assembled in the presence of paclitaxel show moderate binding affinity to and rapid dissociation from tau protein [19].

Breast cancer is a heterogeneous disease with high individual variability as far as response to anticancer drugs is concerned [24]. Biological markers able to predict responsiveness to therapy would allow selection of the most appropriate treatment for each patient. Paclitaxel is generally considered as one of the most active drugs available in the palliative setting of MBC. Predictive markers of paclitaxel efficacy are clearly needed to avoid unnecessary toxicity in nonresponding or resistant patients and to improve the cost-effectiveness of chemotherapy. Although several mechanisms of taxane resistance have been demonstrated in vitro, including overexpression of *p*-glycoprotein and βIII-tubulin isoform, or phosphorylation of bcl-2, no useful markers of paclitaxel resistance have yet been applied in clinical practice [8–18].

As Rouzier et al. also described, low tau expression renders microtubules more vulnerable to paclitaxel and makes breast cancer cells hypersensitive to the drug. They postulated that tau could represent a molecular target for therapy to elevate sensitivity to paclitaxel by inhibiting tau expression in early breast cancer cases. We propose that tau inhibition would also improve the clinical effectiveness of paclitaxel in MBC cases. Although the tau-negative rate was not as frequent among paclitaxel responders compared with previous reports, 60% of cases with tau-negative expression showed favorable clinical response to paclitaxel administration in this study. In addition, 85% of tau-positive cases showed no response to paclitaxel therapy. This observation indicates that high tau expression cases may not benefit from paclitaxel therapy in MBC

Tau contains an imperfect estrogen-response element upstream of the promoter and is an estrogen-induced protein in cultured cells [25–27]. Indeed, we also observed an association between high tau expression and estrogen receptor (ER) positive status. Ten (77%) of 13 cases showing ER-positive expression were tau-positive. In addition, eight (80%) of ten cases showing both ER- and tau-positivity were unresponsive to paclitaxel. This phenomenon may partly explain why ER-positive breast cancers are less sensitive to paclitaxel therapy. Tau alone is an imperfect marker, with 40% of all low tau expression cases showing no effect on paclitaxel susceptibility. It is suggested that there are other mechanisms of resistance that may protect cells from paclitaxel, even if the microtubules are hypervulnerable because of low tau expression.

In conclusion, tau-positive expression may indicate lower susceptibility to paclitaxel treatment than tau-negative expression in MBC. This suggests that tau expression could be used at diagnosis to select patients for whom paclitaxel treatment is likely to be of most benefit. However, this study has some clear limitations in terms of the modest sample size and the heterogeneity of eligible patients (e.g., different numbers and regimens for adjuvant and MBC chemotherapy). Many more cases must be examined before tau expression can be applied to practical clinical treatment.

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