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The association between Akt activation and resistance to hormone therapy in metastatic breast cancer

Eriko Tokunaga[a,*], *Akemi Kataoka*[b], *Yasue Kimura*[a], *Eiji Oki*[a], *Kojiro Mashino*[a], *Kojiro Nishida*[a], *Tadashi Koga*[a], *Masaru Morita*[a], *Yoshihiro Kakeji*[a], *Hideo Baba*[c], *Shinji Ohno*[b], *Yoshihiko Maehara*[a]

[a]*Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan*
[b]*Department of Breast Oncology, National Kyushu Cancer Center, Japan*
[c]*Department of Gastroenterological Surgery, Postgraduate School of Medicine, Kumamoto University, Japan*

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ABSTRACT

In this retrospective study, the relationship between Akt activation and the efficacy of endocrine therapy for metastatic breast cancer was investigated. Thirty-six metastatic breast cancer patients, treated with endocrine therapy, were evaluated for the activation of Akt by an immunohistochemical assessment of the expression of phosphorylated Akt at Ser 473 (pAkt). The relationship between the efficacy of endocrine therapy and Akt activation, HER2 status and hormone receptor expression was also investigated. Of these 36 cases, 12 cases (33.4%) were considered to show a positive pAkt expression. In the pAkt-positive patients, endocrine therapy demonstrated a worse efficacy than in pAkt-negative patients ($P < 0.01$). pAkt positivity was also associated with a poorer objective response ($P < 0.05$). The clinical benefit rate was lower in HER2 positive groups than in HER2 negative group ($P < 0.05$). In addition, the clinical benefit was the smallest in both the HER2 and pAkt-positive patients ($P < 0.01$). Regarding the endocrine agents, the clinical benefit of estrogen deprivation therapy with aromatase inhibitor or luteinising hormone-releasing hormone agosists was significantly lower in the pAkt-positive patients than that in the pAkt-negative ones ($P < 0.05$). In addition, there was a tendency for clinical benefit of selective estrogen receptor modulator to be smaller in the pAkt-positive patients ($P = 0.09$). These findings, therefore, suggest that Akt activation induces endocrine resistance in metastatic breast cancer, irrespective of the kind of endocrine agents that were administered. Our findings suggest that the activation of Akt in the downstream pathway of HER2 plays an important role in the resistance to endocrine therapy for breast cancer. Although our study was small in scope and retrospective in design, our findings suggest that pAkt may be a useful predictor of resistance to endocrine therapy for breast cancer, while also suggesting that the inhibition of Akt may increase the efficacy of endocrine therapy.

* *Corresponding author:* Tel.: +81 92 642 5466; fax: +81 92 642 5482.
E-mail address: eriko@surg2.med.kyushu-u.ac.jp (E. Tokunaga).
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1. Introduction

Breast cancer is the most common cancer in women in the Western World, and the numbers breast cancer patients are also growing year by year in Japan.[1,2] Endocrine therapy was first introduced more than 100 years ago, however, it is still the most effective systemic treatment for patients with hormone receptor-positive breast cancer.

Tamoxifen is the most widely used selective estrogen receptor modulator (SERM) and it has been regarded as the gold standard endocrine therapy for hormone receptor-positive breast cancer.[3] Aromatase inhibitors (AIs) are new drugs which are used for endocrine treatment of post-menopausal breast cancer and they have demonstrated efficacy in patients with breast cancer resistant to anti-estrogens.[4] In addition, third-generation AIs, non-steroidal agents, letrozole and anastrozole, and the steroidal agent exemestane have all demonstrated both efficacy and safety advantages over tamoxifen. The increase in the use of the endocrine agents has resulted in the development of more strategies for the treatment of breast cancer. The major clinical problem in endocrine therapy is tumour resistance, either de novo or acquired during the treatment. About half of all estrogen receptor (ER)-positive tumours are responsive at first presentation to endocrine therapy, however, they eventually become resistant to it with the progression of the disease.[3]

Major clinical trials have shown that the ER status is the strongest and most reliable predictor of the response to endocrine therapy.[5] However, there are limitations in predicting the efficacy of endocrine therapy based on the hormone receptor expression alone. Progesterone receptor (PR) is an estrogen-regulated gene and the presence of PR is an indicator of a functional ER protein and a higher likelihood of a positive response to endocrine therapy.[6] However, about 30% of both ER- and PR-positive tumours remain resistant to endocrine therapy. These findings imply that other factors other than ER and PR are thus involved in resistance to endocrine therapy.

Recently, the cross-talk between signal transduction pathways and ER signalling has been focused on breast cancer etiology and progression.[7] This cross-talk, which occurs at multiple levels, has recently been shown to be associated with endocrine resistance.[8–12] Estrogen-activated membrane ER either directly or indirectly activates membrane tyrosine kinase receptors and this interaction leads to the activation of key secondary signalling messengers and downstream kinase pathways such as ERK/MAPK and PI3K/Akt. These kinases phosphorylate ER at key positions, and in turn, activate both nuclear ER transcriptional activity and promote ER-dependent transcription.[12]

Akt, which is also known as protein kinase B (PKB), is a serine/threonine protein kinase, which is activated by a variety of stimuli, through growth factor receptors, in a phosphoinositide-3-OH kinase (PI3-kinase)-dependent manner.[13] The disruption of normal Akt/PKB signalling occurs frequently in several human cancers, and this enzyme appears to play an important role in cancer progression and cell survival.[13] The mechanisms by which Akt promotes cell survival include phosphorylation of the pro-apoptotic proteins BAD, caspase-9, Forkhead transcription factors and IκB kinase α.[13] In addition, the mammalian target of rapamycin (mTOR) is a downstream effector of the PI3K/Akt signalling pathway that activates p70S6 kinase and 4E-binding protein-1, which in turn regulates the transition G1-S phase of the cell cycle. Breast cancer cell lines with a constitutively activated PI3K/Akt pathway due to HER2 overexpression and/or loss of the PTEN suppressor gene have been shown to be resistant to HER2-, EGFR-targeted therapies and to endocrine therapy with tamoxifen.[14] In addition, breast cancer cell lines with activated Akt are especially sensitive to mTOR antagonism.[15] Therefore, the PI3K/Akt signalling pathway currently attracts considerable attention as a new target for effective therapeutic strategies.

The activation of Akt/PKB has recently been shown to be positively associated with a worse outcome among endocrine-treated breast cancer patients.[16,17] In pre-menopausal patients who were treated with tamoxifen and/or goserelin, the patients with activated Akt were found to be more prone to suffering a relapse with distant metastasis.[16] On the other hand, in post-menopausal patients with a negative status of Akt showed a significant benefit from tamoxifen.[17] Recently, we have also reported Akt activation to be associated with a poor disease-free survival in cases with post-operative hormone therapy.[18] These findings suggest that the status of Akt activation could thus be used as a predictive marker for the sensitivity to endocrine therapy for breast cancer. However, the role of Akt in the resistance to endocrine therapy has not yet been clarified in metastatic breast cancer.

In the present study, we have investigated the relationship between Akt activation and the efficacy of endocrine therapy for metastatic breast cancer. We evaluated the activation of Akt by an immunohistochemical assessment of the expression of phosphorylated Akt (pAkt). pAkt positivity was thus found to be significantly associated with resistance to endocrine therapy. Our results suggest that: (1) Akt activation induces resistance to endocrine therapy; (2) Akt activation thus appears to be useful as a predictive marker of endocrine therapy and; (3) the inhibition of the Akt signalling pathway may improve the efficacy of endocrine therapy for metastatic breast cancer.

2. Patients and methods

2.1. *Patient population and tumour specimens*

A total 36 patients with metastatic breast carcinoma were investigated in this study, and all 36 patients had been treated with endocrine therapy at the Department of Surgery and Science, Kyushu University Hospital, or the Department of Breast Oncology, National Kyushu Cancer Center from 2002 to 2004. Primary human breast carcinoma specimens were obtained and subjected to pathological examinations and immunohistochemical analyses. Informed consent was obtained from all patients prior to tissue acquisition. Clinical data were obtained from medical records. The clinico-pathological features of these patients are described in Table 1.

Table 1 – Clinico-pathological features in the patients

Variables	Number
Menopausal status	
Pre-menopausal	10 (27.8%)
Post-menopausal	26 (72.2%)
Disease sites	
Bone	12
Lung	11
Lymph node	10
Soft tissue	6
Pleura	2
Liver	1
Others	2
Adjuvant therapy	
Chemotherapy	13
Endocrine therapy	9
Chemotherapy and endocrine therapy	9
None	5
ER	
Negative	3 (8.3%)
Positive	33 (91.7%)
PR	
Negative	9 (25.7%)
Positive	26 (74.3%)
Unknown	1
ER/PR	
Positive/negative	9 (25.7%)
Negative/positive	3 (8.6%)
Positive/positive	23 (65.7%)
Positive/unknown	1
HER2	
0, 1+	30 (83.3%)
2+	3 (8.3%)
3+	3 (8.3%)
pAkt	
Negative	24 (66.7%)
Positive	12 (33.3%)

2.2. *Assessment of the efficacy*

After initiating each endocrine therapy, the patients were assessed monthly to evaluate their clinical response. The response categories were defined according to World Health Organization criteria as a complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Both CR and PR were regarded as an objective clinical response. When CR, PR and SD longer than 6 months were obtained, the results were considered to demonstrate a clinical benefit.

2.3. *Immunohistochemistry*

Tissue samples were fixed by immersion in buffered formalin and then embedded in paraffin. Four-micron sections were placed onto charged slides and dried at 60 °C for 1 h. The sections were deparaffinized and hydrated in water. Immunostaining of these paraffin sections was performed using the Ventana Discovery™ automated staining instrument (Ventana Medical Instruments), and hematoxylin (Ventana Medical Instruments) was employed as a nuclear counterstained. Immunostaining was visualized with a streptoavidin peroxidase reaction using DAB as the chromogen (Ventana Medical Instruments). A negative-control reaction with no primary antibody was always performed alongside the reaction-containing sample. Immunostaining was evaluated without any knowledge of the clinical and pathological parameters.

2.4. *Predictive marker analysis*

As predictive markers for endocrine therapy, ER, PR and HER2 were analyzed by immunohistochemical staining. Monoclonal antibodies 6F11 and 1A6 (Ventana Medical Instruments, Tuscon, AR, USA) were used for ER and PR staining. For the HER2 evaluation, the monoclonal antibody, CB11 (Ventana Medical Instruments), was used.[18] ER and PR were considered to be positive if 10% or more of the nuclei in the invasive component of the tumour were stained.[19] HER2 was scored by the widely accepted criteria that assessed the intensity and completeness of membrane staining as previously described.[20,21] The intensity of membrane staining was evaluated according to the following criteria: score 0, none or up to 10% membrane staining; score 1+, partial and/or faint membrane staining present in more than 10% of tumour cells; score 2+, weak to moderate, complete membrane staining present in more than 10% of tumour cells; and score 3+, strong, complete membrane staining present in more than 10% of tumour cells. Scores 0 and 1+ were considered to be normal (i.e., negative for overexpression) and scores 2+ and 3+ were considered positive for HER2 overexpression.

2.5. *Evaluation of Akt activation*

The status of Akt activation was analyzed by the expression of phosophorylated Akt (pAkt). pAkt was detected using polyclonal antibodies against phosphorylated Ser 473 (Cell Signalling Technology, Beverly, MA, USA). A specimen was regarded as positive for pAkt when 10% or more of the cytoplasm of the tumour cells was positively stained.[18]

2.6. *Statistical analysis*

The associations between the categorical variables were assessed by means of the χ^2 tests. The cut-off for significance was set at $P < 0.05$.

3. Results

3.1. *Expression of hormone receptors and HER2 in breast carcinoma tissue specimens*

In this study, 36 primary breast carcinoma specimens, obtained from the patients with metastatic breast cancer, were evaluated. The expressions of estrogen receptor (ER), progesterone receptor (PR) and HER2 were investigated by immunohistochemistry (IHC). ER and PR were positive in 33 cases (91.7%) and 26 cases (74.3%), respectively. Both ER and PR were positive in 23 cases (65.7%). In most of these cases, HER2 was

negative (0 or 1+). HER2-positivity was 8.3% (3 cases) for HER2 2+ and was also 8.3% (3 cases) for HER2 3+ (Table 1).

3.2. *Expression of pAkt in primary breast cancer tissue specimens and the relationship between Akt activation and the HER2 status*

Of these 36 cases, 12 cases (33.3%) were regarded as positive for pAkt expression. Regarding the relationship between pAkt and HER2 expression, pAkt expression was positively correlated with HER2 expression ($P < 0.01$) even though the positivity of HER2 expression was low (Table 2). As a result, in HER2 2+ and 3+ tumours, Akt was more highly activated than HER2-negative tumours.

3.3. *Response to endocrine therapies*

The endocrine therapies received by these patients included the following; aromatase inhibitors (anastrozole or exemestane) in 23 patients, selective estrogen receptor modulator (SERM) (tamoxifen or tremifen) in 15 patients, luteinising hormone-releasing hormone (LHRH) agonist (Goselerin) with or without tamoxifen in seven patients, and medroxyprogesterone acetate (MPA) in one patient. The response data are shown in Table 3. Among 46 therapies in 36 patients, 5 CR and 11 partial responses were achieved. Objective response was observed in 16 cases (34.8%), while clinical benefit was observed in 27 (58.7%) therapies (5 CR, 11 partial responses and 11 long SD).

3.4. *Relationship between the clinical benefits and hormone receptor expression*

First of all, the relationship between clinical benefits and hormone receptor expression was examined. The clinical benefit rate was highest in ER-positive/PR-positive group. However, no statistical differences were seen in the clinical benefit in terms of the hormone receptor expression (Table 4).

Table 2 – Relationship between pAkt and the HER2 status

HER2	*n*	pAkt-negative	pAkt-positive	P-value
		(*n* = 24)	(*n* = 12)	
0, 1+	30	23 (76.7%)	7 (23.3%)	$P < 0.01$
2+	3	1 (20.0%)	2 (66.7%)	
3+	3	0 (0%)	3 (100%)	

3.5. *Relationships between the clinical benefits and the status of HER2 and pAkt*

We next investigated the relationship between HER2 status and clinical efficacy. As described in Table 5, the clinical benefit rate was lower in the HER2 2+ and 3+ groups ($P < 0.05$). As recent studies have suggested that high Akt activity in breast carcinoma is associated with poor prognosis in patients with adjuvant endocrine therapy, we, therefore, hypothesized that pAkt might be associated with poor response to endocrine therapy in metastatic breast cancer. As expected, pAkt positivity was significantly associated with ineffectiveness. In pAkt-positive patients, endocrine therapy had worse efficacy than in pAkt-negative patients ($P < 0.01$) (Table 5). pAkt positivity was also associated with poorer objective response ($P < 0.05$) (Table 6). In addition, the clinical benefit was the

Table 4 – Relationship between clinical benefit and hormone receptor expression

Variables	*n*	Clinical benefits		P-value
		Yes (*n* = 27)	No (*n* = 19)	
ER/PR				
Positive/negative	13	6 (46.2)	7 (53.8)	NS
Negative/positive	4	2 (50.0)	2 (50.0)	
Positive/positive	28	18 (64.3)	10 (35.7)	

Table 5 – Relationship between clinical benefit and the status of HER2 and pAkt

Variables	*n*	Clinical benefits		P-value
		Yes (*n* = 27)	No (*n* = 19)	
HER2				
0, 1+	39	26 (66.7)	13 (33.3)	$P < 0.05$
2+	3	1 (33.3)	2 (66.7)	
3+	4	0 (0)	4 (100.0)	
pAkt				
Negative	28	21 (75.0)	7 (25.0)	$P < 0.01$
Positive	18	6 (33.3)	12 (66.7)	
HER2/pAkt				
Negative/negative	27	20 (74.1)	7 (25.9)	$P < 0.01$
Positive/negative	1	1 (100.0)	0 (0)	
Negative/positive	12	6 (50.0)	6 (50.0)	
Positive/positive	6	0 (0)	6 (100.0)	

Table 3 – Endocrine therapies received by patients and their efficacies

Regimen	*n*	CR	PRes	SD	PD	OR (%)	Clinical benefit (%)
Aromatase inhibitors	23	4	5	5	9	9 (39.1)	14 (60.9)
LHRH agonist	7	0	2	1	4	2 (28.6)	3 (42.9)
SERM	15	1	4	4	6	5 (33.3)	9 (60.0)
Methylprogesterone acetate	1	0	0	1	0	0 (0)	1 (100)
Total	46	5	11	11	19	16 (34.8)	27 (58.7)

CR, complete response; PRes, partial response; SD, stable disease; PD, progressive disease; OR, objective response is CR + PRes; Clinical benefit is CR + PRes + SD.

Table 6 – Relationship between objective response and Akt activation

pAkt	CR, PR	SD, PD	P-value
	(*n* = 16, 34.8%)	(*n* = 30, 65.2%)	
Negative	13	15	$P < 0.05$
Positive	3	15	

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

smallest in the both HER2 and pAkt-positive patients ($P < 0.01$) (Table 5).

3.6. *Relationships between the clinical benefits and the status of HER2 and pAkt by endocrine agents*

We next evaluated whether the kind of endocrine therapies influenced the association between efficacy and expression of HER2 or pAkt. We divided the different endocrine therapies into estrogen deprivation therapy such as aromatase inhibitors (AIs) or LHRH agonist and SERM (Table 7). In HER2 2+ and 3+ patients, the clinical benefit rate of AI or LHRH agonist was significantly lower than that in HER2 0 or 1+ patients ($P < 0.01$), although the number of the patients of HER2 2+ and 3+ was quite small (Table 7). On the other hand, no association was observed between the HER2 status and clinical efficacy of SERM, probably due to paucity of patients with HER2 overexpression. We then investigated the association between Akt activation and the clinical benefit by endocrine agents. In pAkt-positive patients, the clinical benefit rate of estrogen deprivation therapy, AI or LHRH, was significantly lower than that in pAkt-negative patients ($P < 0.05$). In addition, the clinical benefit of SERM tended to be smaller in the pAkt-positive patients ($P = 0.09$) (Table 7). These findings suggest that Akt activation induces endocrine resistance in metastatic breast cancer irrespective of the kind of endocrine agents that were administered.

Table 7 – Relationships between the clinical benefits and the status of HER2 and pAkt according to the endocrine agent

Variables	*n*	Clinical benefits		P-value
		Yes (*n* = 27)	No (*n* = 19)	
HER2				
AI or LH-RH agonist				
0, 1+	25	17 (68.0)	8 (32.0)	$P < 0.01$
2+	2	0 (0)	2 (100.0)	
3+	3	0 (0)	3 (100.0)	
SERM				
0, 1+	14	9 (64.3)	5 (35.7)	NS
3+	1	0 (0)	1 (100.0)	
pAkt				
AI or LH-RH agonist				
Negative	16	12 (75.0)	4 (25.0)	$P < 0.05$
Positive	14	5 (35.7)	9 (64.7)	
SERM				
Negative	11	8 (72.7)	3 (27.3)	$P = 0.09$
Positive	4	1 (25.0)	3 (75.0)	

4. Discussion

As far as we know, this is the first report to show an association between Akt activation and the efficacy of endocrine therapy in metastatic breast cancer. Akt/PKB is a serine/threonine kinase, which is a downstream effector of PI3K. Major functions of the PI3K/Akt signal pathway includes the promotion of growth-factor-mediated cell growth, proliferation, migration and survival.[13] Because the activation of the PI3K/Akt pathway induces resistance to the apoptotic response, the inhibition of this pathway is now considered to be a promising strategy to improve the effect of therapies for various kinds of cancers (reviewed in [22].)

Recent studies suggest high Akt activity in breast carcinoma to be associated with poor prognosis in patients with adjuvant endocrine therapy.[16–18] Perez-Tenorio and colleagues revealed that pAkt-positive patients were more prone to suffering a relapse with distant metastasis in a study of the pre-menopausal patients who were treated with tamoxifen and/or goserelin.[16] On the other hand, in a study of post-menopausal breast cancer patients, the benefit from tamoxifen was analyzed in estrogen receptor-positive patients.[17] Patients with a negative status of Akt showed a significant benefit from tamoxifen, whereas there was no significant benefit from tamoxifen in patients with positive Akt status.[17] In addition, we recently reported that pAkt positivity was associated with poor disease-free survival in cases with post-operative hormone therapy.[18]

We next investigated whether Akt activation had any impact on the response to endocrine therapy for metastatic breast cancer. In the present study, we analyzed 36 cases of metastatic breast cancer that had been treated with 46 endocrine therapies. In terms of the relationship between Akt activation and the efficacy of endocrine therapy, the clinical benefit rate was significantly lower in the pAkt-positive patients ($P < 0.01$) (Table 5). In addition, HER2 overexpression was associated with a lack of effectiveness of endocrine therapy, although the number of the patients with HER2 overexpression was too small (Table 5). However, this finding could have been expected based on previous experimental and clinical reports.[10,11,16,17,23]

We thereafter investigated whether the association between pAkt and resistance to endocrine therapy differed depending on the endocrine therapy agent. Up to now, the activation of Akt has been reported to be associated with resistance to anti-estrogen such as tamoxifen, however, the relationship between Akt activation and the effect of AIs or LHRH agonist has not yet been elucidated. Interestingly, pAkt-positivity or HER2 overexpression was significantly higher in the non-effective cases than that in effective cases with AIs or LHRH agonist, which are both types of estrogen depletion therapy ($P < 0.01$, $P < 0.05$) (Table 7). In experimental studies, estradiol has been shown to rapidly activate PI3K/Akt through the HER2 pathway[24] and a constitutively active Akt mutant mimics the effect of estrogen in the absence of the estrogen receptor ligand.[25] These results

suggest that breast cancer cells with activated Akt can survive under estrogen suppression by either AI or LHRH agonist. In terms of the relationship between HER2 overexpression and sensitivity to AI, a randomized trial of neoadjuvant therapy showed that, in a subset of ER-positive, epidermal growth factor receptor-positive and/or HER2-positive, letrozole was significantly more effective than tamoxifen.[19] However, in the metastatic setting, endocrine therapy has recently been shown to be less effective in patients with HER2-positive tumours irrespective of the drugs administered.[26] Our findings regarding the relationships between HER2 overexpression and sensitivity to estrogen depletion therapy are thus consistent with this meta-analysis in the metastatic setting.[26] In addition, we herein demonstrated that endocrine therapy is less effective in patients with Akt-activated breast cancer irrespective of the endocrine agents administered.

One possible mechanism for endocrine resistance in Akt-activated cells that we propose is illustrated in Fig. 1. When Akt is activated for any reason, it promotes the ER target gene expression even in the absence of estrogen or when being treated with tamoxifen. The estrogen independent tumour growth may also partly be associated with such resistance.

In this study, we have demonstrated that Akt/PKB activation was significantly associated with a poor response to endocrine therapy for metastatic breast cancer. The results of this study suggest that an inhibition of the Akt signalling pathway may improve the efficacy of the endocrine therapy for metastatic breast cancer. In fact, there are some currently ongoing or planned phase II/III clinical trials of endocrine therapy, either with or without signal transduction inhibitors, in locally advanced or metastatic breast cancer.[8] In combination with AIs and tamoxifen, monoclonal antibodies such as trastuzumab, and tyrosine kinase inhibitors such as gefinitib or lapanitib, and mTOR inhibitors, CCI-779 or RAD001 were recruited in these trials. Trastuzumab and tyrosine kinase inhibitors have a potency to inhibit Akt activity, and the inhibition of mTOR, which is downstream of Akt, can also lead to the inhibition of Akt signalling. The data obtained from these studies will hopefully lead to an improvement in the treatment of breast cancer patients.

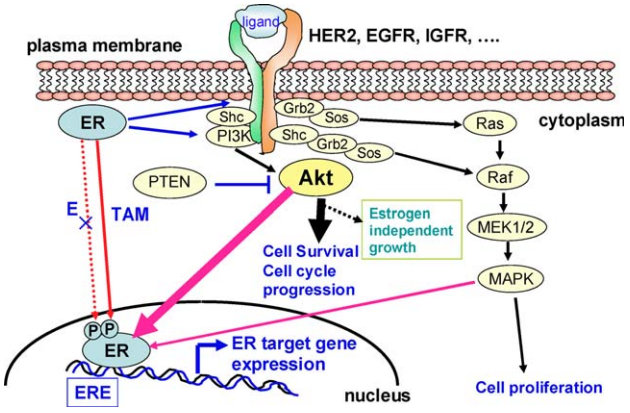

Fig. 1 – Possible mechanism for endocrine resistance in Akt-activated cells. In breast cancer cells, stimuli such as the activation of membrane tyrosine kinase and the loss of PTEN function are known to lead to Akt activation. Activated Akt promotes ER target gene expression even in the absence of estrogen (under treatment with aromatase inhibitors or LHRH agonists) or while being treated with tamoxifen, which induces cell growth. Estrogen independent tumour growth of Akt-activated cancer cells may partly be associated with such resistance.

In conclusion, this study suggests that pAkt may be a useful predictor of resistance to endocrine therapy for breast cancer, while also suggesting that the inhibition of Akt may increase the efficacy of endocrine therapy, although our study was small in scope and retrospective in design. Similar examinations in well-designed, larger-scale prospective studies should provide us more valuable findings in the future.

Conflict of interest statement

The authors of this paper have no financial or personal relationships that could bias this work.

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