BMB
Reports

Fibronectin expression is upregulated by PI-3K/Akt activation in tamoxifen-resistant breast cancer cells

Daeun You[1,#], Seung Pil Jung[4,#], Yisun Jeong[1], Soo Youn Bae[4], Jeong Eon Lee[1,2,3,] & Sangmin Kim[3,*]*

[1]Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Departments of [2]Surgery, [3]Breast Cancer Center, Samsung Medical Center, Seoul 06351, [4]Division of Breast and Endocrine Surgery, Department of Surgery, Korea University Hospital, Korea University College of Medicine, Seoul 02852, Korea

Fibronectin (FN) plays important roles in the EMT in a variety of cancer cell types. However, the mechanism by which FN expression is regulated in tamoxifen-resistant (TamR) breast cancer cells has not yet been fully elucidated. Aberrant FN expression was associated with poor prognosis in patients with luminal type A breast cancer. In addition, FN was upregulated in TamR cells. To investigate the mechanism by which FN expression is regulated, we assessed the levels of phosphorylated Akt, JNK, and STAT3 and found that they were all increased in TamR cells. Induction of FN expression was dampened by LY294002 or AKT IV in TamR cells. Furthermore, FN expression was increased by constitutively active (CA)-Akt overexpression in tamoxifen-sensitive MCF7 (TamS) cells and colony formation of TamR cells was blocked by AKT IV treatment. Taken together, these results demonstrate that FN expression is upregulated through the PI-3K/Akt pathway in tamoxifen-resistant breast cancer cells. [BMB Reports 2017; 50(12): 615-620]

INTRODUCTION

Breast cancer is the most common cancer in women and the second most common cause of cancer death, accounting for nearly 15% of all cancer-related deaths in women (1). Patients with ER-α-positive breast cancer comprise approximately 70% of all patients with breast cancer (2) and have a good prognosis. Specifically, 85% of all patients with ER-α-positive breast cancer survive more than five years after diagnosis. This good overall survival rate is due to multiple endocrine

*Corresponding authors. Sangmin Kim, Tel: +82-2-2148-7312; Fax: +82-2-3410-6982; E-mail: sangmin3005.kim@samsung.com, Jeong Eon Lee, Tel: +82-2-3410-0260; Fax: +82-2-3410-6982; E-mail: paojlus@hanmail.net
[#]These authors contributed equally to this work.

therapies, including tamoxifen, fulvestrant, and letrozole (2, 3). Although tamoxifen is still the most frequently used selective ER modulator, the effectiveness of tamoxifen therapy is limited in premenopausal women with ER-positive breast cancer (4). Endocrine therapy resistance can arise via several mechanisms, including loss of ERα, induction of abnormal estradiol levels, and alterations of coregulatory proteins such as AIB1 and HDAC (5-7). Therefore, many studies have sought to identify beneficial therapeutic targets that would eliminate the development of drug resistance.

FN is an extracellular matrix glycoprotein that plays an important role in cell-cell adhesion, cell migration, wound healing, and metastasis (8, 9). Abnormal FN expression is associated with poor prognosis in various types of cancer, including breast cancer (9, 10). FN expression is regulated by a variety of growth factors including EGF, TGF-β, and glucocorticoid (10-12). The human FN gene promoter contains specific DNA binding sites, such as TATA box, SP-1, NF-1, and CRE binding sites for nuclear transcription factors that are involved in the modulation of FN transcription (13, 14). Recently, we reported that EGF- and HER2-induced FN expression in breast cancer cells were both significantly decreased by UO126 (9). However, the mechanism by which FN expression is regulated in tamoxifen-resistant breast cancer cells is not fully understood.

The aim of this study was to investigate the mechanism by which FN expression is regulated in TamR cells. We found that aberrant FN expression was associated with poor prognosis in patients with luminal type A breast cancer. We also found that FN expression was significantly higher in TamR cells than in TamS cells. Furthermore, we found that FN expression was regulated by the PI-3K/Akt pathway in TamR cells.

RESULTS

FN expression is associated with poor prognosis in patients with luminal type A breast cancer
Previously, Bae *et al.* reported that FN expression was associated with tumor aggressiveness and poor clinical outcomes in patients with invasive breast cancer (15). Here, we also evaluated whether FN expression was associated with

prognosis. To this end, we analyzed DNA microarray-based gene expression data from patients with luminal type A breast cancer using the KM plotter online tool (http://kmplot.com/ breast). As shown in Fig. 1A, patients with breast cancer with high FN expression had poor prognoses. Patients with high FN expression showed shorter relapse-free survival (P = 0.023) than patients with luminal type A breast cancer with low FN expression (Fig. 1A). However, FN expression was not associated with prognosis in patients with luminal type B breast cancer (Fig. 1A).

To assess FN expression and to determine the relationship of FN expression with tamoxifen in luminal type A breast cancer cells, we used TamS and TamR breast cancer cells. Recently, we reported that these two cell types exhibit morphological differences. TamR cells appear in scattered, loosely-packed colonies, and have many branches similar to those of mesenchymal cells (16). The levels of FN mRNA and protein expression were significantly higher in TamR cells (Fig. 1B); specifically, the FN mRNA expression level was 21.1 $\pm$ 0.89-fold that of control cells (Fig. 1B). In contrast, E-cadherin expression was lower in TamR cells (Fig. 1B). Therefore, these results indicate that aberrant FN expression is associated with poor prognosis in luminal type A breast cancer.

Akt activity is high in tamoxifen-resistant breast cancer cells

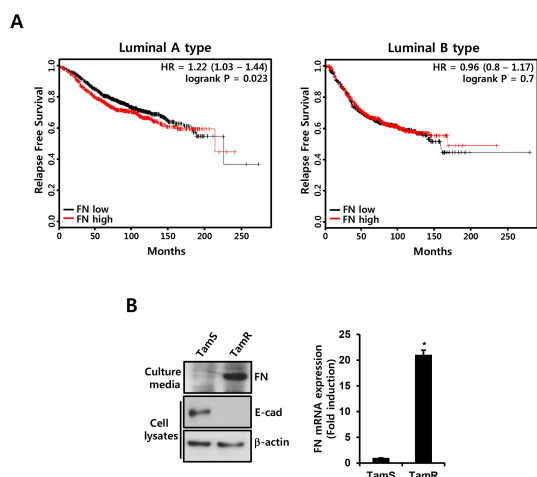We next investigated FN expression-regulated signaling molecules in TamR cells by analyzing the phosphorylation of several signaling molecules including Akt, JNK, and STAT3 in TamS and TamR cells. As shown in Fig. 2A, the levels of phosphorylated Akt, JNK, and STAT3 were dramatically higher in TamR cells compared with those in TamS cells. We also investigated FN expression levels in the presence of the tumor promoter PMA. As shown in Fig. 2B, PMA triggered Akt and JNK phosphorylation in MCF7 cells. In addition, the basal protein levels of FN were significantly increased by PMA treatment (Fig. 2C) in wild type MCF7 cells. The JNK inhibitor SP600125 did not affect FN expression (data not shown). Based on these results, we suggest that Akt activity is directly or indirectly associated with the regulation of FN expression.

FN expression is regulated by a PI-3K/Akt-dependent pathway in tamoxifen-resistant breast cancer cells

To verify the mechanism by which FN expression is regulated, we treated TamR cells with LY294002 for 24 h. The basal protein level of FN was decreased by LY294002 in both culture media and cell lysates (Fig. 3A). As expected, phosphorylated Akt was not detectable (Fig. 3A). In addition, we investigated the effect of LY294002 on PMA-induced FN expression. As shown in Fig. 3B, PMA significantly increased the mRNA and protein levels of FN in wild type MCF7 cells. Furthermore, the basal level of FN expression was decreased by LY294002 in MDA-MB231 TNBC cells (Fig. 3C). These results demonstrate that a PI-3K/Akt-dependent pathway is associated with the regulation of FN expression in breast cancer cells.

Akt activity plays an important role in FN expression and TamR cell growth

Next, we investigated whether Akt activity was directly

Fig. 1. FN expression is associated with poor prognosis in patients with luminal type A breast cancer. (A) Relapse-free survival. FN expression data were obtained from a public database (http:// kmplot.com/breast). The clinical value of FN was analyzed by generating a Kaplan-Meier survival plot for patients with luminal A and B type breast cancer. (B) The levels of FN mRNA and protein expression were analyzed by real-time PCR and Western blotting, respectively. Results are representative of three independent experiments. Data are presented as means $\pm$ SEMs. *P < 0.01 vs. TamS cells.
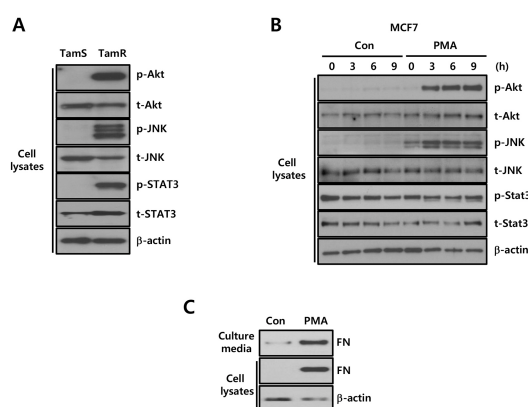

Fig. 2. Akt activity is increased in tamoxifen-resistant breast cancer cells. (A) The levels of p- and t-Akt, JNK, and STAT3 were analyzed by Western blotting. (B, C) After serum starvation, MCF7 cells were treated with 10 nM PMA for the indicated times. (C) FN and β-actin expression in cell culture media and whole cell lysates were analyzed via Western blotting. Results are representative of three independent experiments. Con, Control.
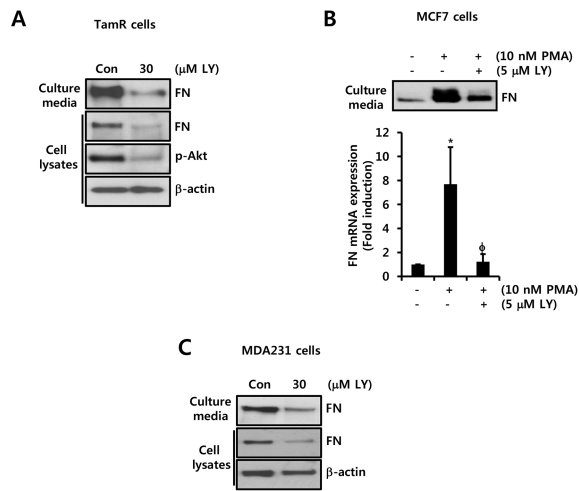
Fig. 3. FN expression is regulated through a PI-3K/Akt-dependent pathway in tamoxifen-resistant breast cancer cells. (A) TamR cells were treated with 30 μM LY for 24 h. (B) MCF7 cells were serum starved for 24 h, pretreated with 5 μM LY for 30 min, and then treated with 10 nM PMA for 24 h. After 24 h, cell lysates and culture media were harvested for detection of FN mRNA and protein expression. (C) MDA-MB231 cells were treated with 30 μM LY for 24 h. The protein levels of FN in cell lysates and culture media were analyzed via Western blotting. Results are representative of three independent experiments. Data are presented as means ± SEMs. *P < 0.05 vs. control, $^{\phi}$P < 0.05 vs. PMA-treated cells. Con, Control; LY, LY294002.

associated with FN expression in TamS cells. We transfected cells with CA-Akt for 24 h and further incubated cells in serum-free medium for 24 h. Next, we harvested cell lysates and culture media for detection of FN mRNA and protein expression. As shown in Fig. 4A, TamS cells overexpressing CA-Akt had 2.27 ± 0.44-fold higher levels of FN compared to control cells (Fig. 4A). In contrast, aberrant FN induction was decreased by AKT IV in TamR cells (Fig. 4B). As a positive control, we examined the effect of AKT IV on FN expression in TNBC cells. Basal FN expression was not detected after AKT IV treatment (Fig. 4C). These results demonstrate that Akt activity plays an important role in the regulation of FN expression in TamR cells.

Finally, we used a soft agar colony formation assay to test whether AKT IV affects the clonogenic potential of TamR cells. As shown in Fig. 4D, anchorage-independent growth of TamR cells treated with AKT IV was observed, as evidenced by the significantly reduced number of colonies and colony size compared with untreated cells. In addition, the number of TamR cells did not increase after AKT IV treatment, in contrast to vehicle-treated TamR cells (Fig. 4E). These results indicate that Akt activity is important for maintaining anchorage-independent growth of tamoxifen-resistant breast cancer cells.
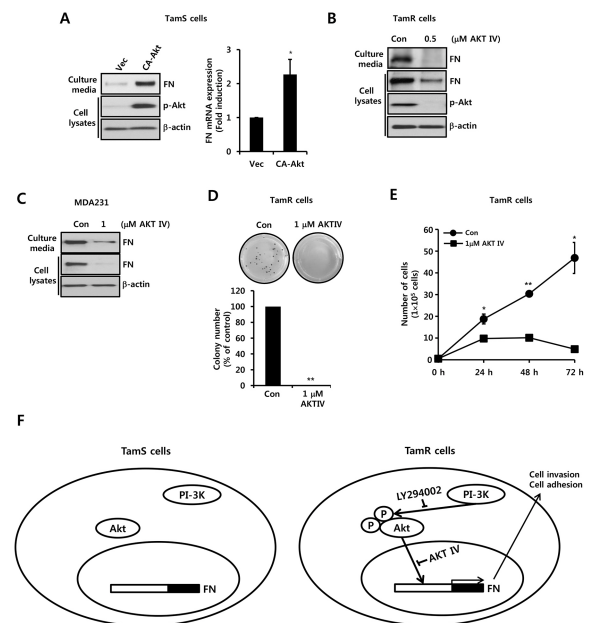

Fig. 4. Akt activity plays an important role in FN expression and TamR cell growth. (A) TamS cells were transfected with adenoviral vectors and CA-Akt for 24 h and then further incubated in serum-free medium for 24 h. (B) TamR cells were treated with 0.5 μM AKT IV for 24 h. (C) After serum starvation for 24 h, MDA-MB231 cells were treated with or without 1 μM AKT IV for 24 h under serum-free conditions. The protein levels of FN, p-Akt, and β-actin were analyzed by Western blotting. (D) TamR cells were seeded on 6-well soft agar plates with or without 1 μM AKT IV and incubated for 2 weeks. After 2 weeks, viable colonies were stained with 0.01% crystal violet. (E) TamR cells were treated with or without 1 μM AKT IV for the indicated time periods, after which cells were counted using a Countess™ Automated Cell Counter. (F) Schematic model of this study. Results are representative of three independent experiments. Data are presented as means ± SEMs. *P < 0.05, **P < 0.01 vs. control. Con, Control.

DISCUSSION

Although endocrine therapies have been used in the treatment of hormone-dependent breast cancer, the majority of patients ultimately relapse and experience disease progression (17). Tamoxifen is a systemic treatment for ER-positive breast cancer. Acquisition of tamoxifen resistance significantly augments the EGFR signaling pathway and increases the metastatic ability of resistant cells (16). In addition, patients with ER (+) metastatic breast cancer with HER-2 amplification and HER-1 expression are moderately less responsive to tamoxifen (18). However, the mechanism of tamoxifen resistance is not fully understood. Here, we investigated the mechanism by which FN expression is regulated, because FN expression has been previously linked to tamoxifen resistance-related genes in breast cancer cells.

FN plays a pivotal role in cell adhesion, invasion, and oncogenic transformation (19). Moreover, FN expression has been correlated with tumor aggressiveness and poor clinical outcomes in patients with invasive breast cancer (15). FN expression has been detected in circulating tumor cells from patients with breast cancer (20). Zheng *et al.* proposed that FN causes epithelial cells to become refractory to tamoxifen by binding to β1 integrin (21, 22). Consistent with these reports, we found that patients with luminal type A breast cancer with high levels of FN showed shorter relapse-free survival. Furthermore, basal FN expression was significantly higher in established TamR cells than in TamS cells. These results demonstrate that the level of FN expression may be associated with endocrine resistance in breast cancer.

Several studies have demonstrated that various transcription factors such as NF-κB and AP-1 are involved in FN synthesis (23). High glucose upregulates FN protein synthesis via NF-κB and AP-1 in endothelial cells (24). Moreover, Lee *et al.* reported that PMA induces transcription of FN in hepatoma cells via the PKC pathway (23). As a positive control, we also treated MCF7 breast cancer cells with PMA and found that PMA-induced FN expression was decreased by LY294002. Furthermore, basal FN expression was significantly decreased by LY294002 treatment in both TamR and TNBC cells. In contrast, FN expression was increased in TamS cells over-expressing CA-Akt. Therefore, these data demonstrate that the PI-3K/Akt pathway plays an important role in regulating FN expression in TamR cells.

The PI-3K/Akt pathway is the most frequently altered pathway in human cancer. Common alterations include mutation and/or amplification of genes encoding the PI-3K catalytic subunits and regulatory subunits (25-27), as well as loss of the lipid phosphatases PTEN and INPP4B (28, 29). Activation of PI3K/Akt has been shown to confer resistance to antiestrogens in various models of breast cancer, including PTEN-deficient cells and mutant AKT1-overexpressing cells (30). Consistent with these reports, we also found that the phosphorylation level of Akt was significantly higher in TamR cells. Furthermore, anchorage-independent growth of TamR cells was completely prevented by a specific Akt inhibitor. Therefore, these data demonstrate that PI-3K inhibitors and Akt inhibitors are promising therapeutic drugs for overcoming tamoxifen resistance.

As shown in Fig. 4F, we explored the mechanism by which FN is regulated in TamR cells. Abnormal FN induction was associated with poor prognosis in patients with luminal type A breast cancer. Furthermore, basal FN expression was significantly higher in TamR cells compared with TamS cells. We also observed that the level of phosphorylated Akt was significantly higher in TamR cells. Furthermore, basal FN expression was increased by CA-Akt overexpression in TamS cells. In contrast, this elevated FN expression was decreased by treatment with the Akt inhibitor AKT IV in TamR cells. In addition, anchorage-independent growth of TamR cells was decreased by AKT IV treatment. Taken together, these data demonstrate that abnormal FN induction is mediated by an Akt-dependent pathway in TamR cells. Thus, the potential of PI-3K/Akt pathway regulation to mitigate endocrine resistance in breast cancer should be further investigated.

MATERIALS AND METHODS

Reagents
Dulbecco's modified Eagle's medium (DMEM) and phenol red-free DMEM were purchased from Thermo Scientific (Hemel Hempstead, UK). Fetal bovine serum (FBS) was purchased from Hyclone (Logan, UT, USA). 4-Hydroxytamoxifen (4-OHT) was purchased from Sigma (St. Louis, MO, USA). LY294002 was purchased from Tocris (Ellisville, MO, USA). AKT IV, secondary HRP-conjugated antibodies, and mouse monoclonal anti-β-actin antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Antibodies against total (t) and phospho (p)-Akt, STAT3, and JNK were purchased from Cell Signaling Technology (Beverly, MA). Anti-FN antibodies were purchased from Abcam (Cambridge, United Kingdom). West-Q Chemiluminescent Substrate Plus kit ware obtained from Genedepot (Barker, TX, USA).

Analysis of public database expression data
Expression data were downloaded from a public database [Kaplan-Meier plotter database (http://kmplot.com/breast)] (31). The clinical value of FN levels in patients with luminal type A and B breast cancer was determined by Kaplan-Meier analysis. Hazard ratios with 95% confidence intervals and log-rank P values were calculated.

Establishment of tamoxifen-resistant MCF-7 breast cancer cells
Briefly, MCF-7 cells were washed with PBS, after which the culture medium was changed to phenol red-free DMEM containing 10% charcoal-stripped steroid-depleted FBS and 0.1 μM 4-OHT. The cells were continuously exposed to this treatment regimen for 2 weeks, after which the 4-OHT concentration was increased gradually up to 3 μM over a 9-month period. Initially, cell growth was reduced. However, after exposure to the medium for 9 months, cell growth gradually increased, indicating the establishment of tamoxifen-resistant cells (32).

Cell culture and drug treatment
TamS and TamR breast cancer cells were cultured in DMEM supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Cells were grown in a humidified atmosphere with 5% CO_2 at 37°C. In the drug treatment experiment, TamR cells were serum starved for 24 h and then treated with specific inhibitors at the indicated concentrations for 24 h.

Western blotting

Cell lysates were prepared to detect t- and p-Akt, STAT3, JNK, FN, and β-actin expression. Equal amounts of proteins (50 μg) were boiled for 5 min in Laemmli sample buffer and then electrophoresed on 8% SDS-PAGE gels. The separated proteins were transferred to PVDF membranes, after which the membranes were blocked with 10% skim milk in Tris-buffered saline (TBS) containing 0.01% Tween-20 (TBS/T) for 15 min. The blots were washed three times in TBS/T and then incubated with antibodies against t- or p-Akt, STAT3, JNK, FN, or β-actin in TBS/T buffer at 4°C overnight. The blots were washed three times in TBS/T and subsequently incubated with secondary HRP-conjugated antibodies in TBS/T buffer. After 1 h incubation at room temperature (RT), blots were washed three times in TBS/T. Immunoreactive bands were detected using the West-Q Chemiluminescent Substrate Plus kit.

Real-time polymerase chain reaction (PCR)

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Isolated RNA samples were then used for RT-PCR. Total RNA (1 μg) was reverse-transcribed into cDNA in 20 μl reaction volumes using a first-strand cDNA synthesis kit for RT-PCR, according to the manufacturer's instructions (MBI Fermentas, Hanover, MD, USA). Gene expression levels were quantified by real-time PCR using a SensiMix SYBR kit (Bioline Ltd., London, UK) and 100 ng of cDNA per reaction. The primer sequences used for this analysis were as follows: human FN (forward, 5′-CCA CCC CCA TAA GGC ATA GG-3′; reverse, 5′-GTA GGG GTC AAA GCA CGA GTC ATC-3′) and GAPDH as an internal control (forward, 5′-ATT GTT GCC ATC AAT GAC CC-3′; reverse, 5′-AGT AGA GGC AGG GAT GAT GT-3′). An annealing temperature of 60°C was used for all primers. PCR was performed in a standard 384-well plate format with an ABI 7900HT real-time PCR detection system (Foster City, CA, USA). For data analysis, the raw threshold cycle (C_T) value was first normalized to the housekeeping gene for each sample to obtain a ΔC_T value. The normalized ΔC_T value was then calibrated to control cell samples to obtain $\Delta\Delta C_T$ values.

Adenovirus induction

Empty (Lac Z) and adenoviral human constitutively active (CA) human AKT cDNA was a gift from Dr. Hyunil Ha (Korea Institute of Oriental Medicine, Daejeon, Korea). The recombinant adenovirus expressing human CA-Akt was produced in 293A cells. TamS cells were transfected with each construct for 24 h, after which cells were incubated for 24 h in fresh culture medium. Vec and CA-Akt-overexpressing TamS cells were further incubated for 24 h in serum-free culture medium. After 24 h of incubation, cell lysates and culture media were harvested and analyzed for FN, β-actin, and p-Akt expression.

Soft agar colony formation assay

TamR breast cancer cells were seeded at a density of 5 × 10^4 cells/well in 6-well plates in growth medium containing 0.7% agar (1.5 ml/well). Cells were seeded on top of a layer of growth medium containing 1.4% agar (2 ml/well). Next, growth medium (500 μl) with 10% FBS was added on top of the agar. In addition, 1 μM AKT IV was added on top of the agar for some of the plates. Cells were plated and cultured in a 37°C incubator for 2 weeks. After 2 weeks, viable colonies were stained with 0.01% crystal violet and observed using a CK40 inverted microscope (Olympus, Tokyo, Japan).

Statistical analysis

Statistical significance was determined using Student's *t*-test. Results are presented as means ± SEMs. All P values are two-tailed; differences were considered statistically significant for P < 0.05. Statistical analyses were performed using Microsoft Excel.

ACKNOWLEDGEMENTS

CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Beiki O, Hall P, Ekbom A and Moradi T (2012) Breast cancer incidence and case fatality among 4.7 million women in relation to social and ethnic background: a population-based cohort study. Breast Cancer Res 14, R5
2. Lim E, Metzger-Filho O and Winer EP (2012) The natural history of hormone receptor-positive breast cancer. Oncology (Williston Park) 26, 688-694, 696
3. Regan MM, Neven P, Giobbie-Hurder A et al (2011) Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: the BIG 1-98 randomised clinical trial at 8.1 years median follow-up. Lancet Oncol 12, 1101-1108
4. Colditz GA (1998) Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. J Natl Cancer Inst 90, 814-823
5. Paridaens R, Sylvester RJ, Ferrazzi E, Legros N, Leclercq G and Heuson JC (1980) Clinical significance of the quantitative assessment of estrogen receptors in advanced breast cancer. Cancer 46, 2889-2895
6. Ravdin PM, Fritz NF, Tormey DC and Jordan VC (1988)

Endocrine status of premenopausal node-positive breast cancer patients following adjuvant chemotherapy and long-term tamoxifen. Cancer Res 48, 1026-1029

7. Anzick SL, Kononen J, Walker RL et al (1997) AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277, 965-968

8. Ruoslahti E (1988) Fibronectin and its receptors. Annu Rev Biochem 57, 375-413

9. Jeon M, Lee J, Nam SJ, Shin I, Lee JE and Kim S (2015) Induction of fibronectin by HER2 overexpression triggers adhesion and invasion of breast cancer cells. Exp Cell Res 333, 116-126

10. Kim S, Jeon M, Lee J et al (2014) Induction of fibronectin in response to epidermal growth factor is suppressed by silibinin through the inhibition of STAT3 in triple negative breast cancer cells. Oncol Rep 32, 2230-2236

11. Dean DC, Newby RF and Bourgeois S (1988) Regulation of fibronectin biosynthesis by dexamethasone, transforming growth factor beta, and cAMP in human cell lines. J Cell Biol 106, 2159-2170

12. Blatti SP, Foster DN, Ranganathan G, Moses HL and Getz MJ (1988) Induction of fibronectin gene transcription and mRNA is a primary response to growth-factor stimulation of AKR-2B cells. Proc Natl Acad Sci U S A 85, 1119-1123

13. McDonald JA (1988) Extracellular matrix assembly. Annu Rev Cell Biol 4, 183-207

14. Dean DC (1989) Expression of the fibronectin gene. Am J Respir Cell Mol Biol 1, 5-10

15. Bae YK, Kim A, Kim MK, Choi JE, Kang SH and Lee SJ (2013) Fibronectin expression in carcinoma cells correlates with tumor aggressiveness and poor clinical outcome in patients with invasive breast cancer. Hum Pathol 44, 2028-2037

16. Kim S, Lee J, Oh SJ, Nam SJ and Lee JE (2015) Differential effect of EGFR inhibitors on tamoxifen-resistant breast cancer cells. Oncol Rep 34, 1613-1619

17. Ali S and Coombes RC (2002) Endocrine-responsive breast cancer and strategies for combating resistance. Nat Rev Cancer 2, 101-112

18. Arpino G, Green SJ, Allred DC et al (2004) HER-2 amplification, HER-1 expression, and tamoxifen response in estrogen receptor-positive metastatic breast cancer: a southwest oncology group study. Clin Cancer Res 10, 5670-5676

19. Ioachim E, Charchanti A, Briasoulis E et al (2002) Immunohistochemical expression of extracellular matrix components tenascin, fibronectin, collagen type IV and laminin in breast cancer: their prognostic value and role in tumour invasion and progression. Eur J Cancer 38, 2362-2370

20. Raimondi C, Gradilone A, Naso G et al (2011) Epithelial-mesenchymal transition and stemness features in circulating tumor cells from breast cancer patients. Breast Cancer Res Treat 130, 449-455

21. Zheng LH, Zhao YH, Feng HL and Liu YJ (2014) Endocrine resistance in breast cancer. Climacteric 17, 522-528

22. Oh S, Kim H, Nam K, Shin I (2017) Glut1 promotes cell proliferation, migration and invasion by regulating epidermal growth factor receptor and integrin signaling in triple-negative breast cancer cells. BMB Rep 50, 132-137

23. Lee BH, Kim MS, Rhew JH, Park RW, de Crombrugghe B and Kim IS (2000) Transcriptional regulation of fibronectin gene by phorbol myristate acetate in hepatoma cells: a negative role for NF-kappaB. J Cell Biochem 76, 437-451

24. Chen S, Mukherjee S, Chakraborty C and Chakrabarti S (2003) High glucose-induced, endothelin-dependent fibronectin synthesis is mediated via NF-kappa B and AP-1. Am J Physiol Cell Physiol 284, C263-272

25. Jaiswal BS, Janakiraman V, Kljavin NM et al (2009) Somatic mutations in p85alpha promote tumorigenesis through class IA PI3K activation. Cancer Cell 16, 463-474

26. Campbell IG, Russell SE, Choong DY et al (2004) Mutation of the PIK3CA gene in ovarian and breast cancer. Cancer Res 64, 7678-7681

27. Park S, Sim H, Lee K (2016) Rapamycin-resistant and torin-sensitive mTOR signaling promotes the survival and proliferation of leukemic cells. BMB Rep 49, 63-68

28. Perez-Tenorio G, Alkhori L, Olsson B et al (2007) PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. Clin Cancer Res 13, 3577-3584

29. Fedele CG, Ooms LM, Ho M et al (2010) Inositol polyphosphate 4-phosphatase II regulates PI3K/Akt signaling and is lost in human basal-like breast cancers. Proc Natl Acad Sci U S A 107, 22231-22236

30. Miller TW, Balko JM and Arteaga CL (2011) Phosphatidylinositol 3-kinase and antiestrogen resistance in breast cancer. J Clin Oncol 29, 4452-4461

31. Gyorffy B, Lanczky A, Eklund AC et al (2010) An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. Breast Cancer Res Treat 123, 725-731

32. Choi HK, Yang JW, Roh SH, Han CY and Kang KW (2007) Induction of multidrug resistance associated protein 2 in tamoxifen-resistant breast cancer cells. Endocr Relat Cancer 14, 293-303