**Table 4** Histological features of Early growth responsive gene 3 (*Egr3*)-expressing MCF-7 cells injected into athymic mice

| | Eg-11 ($n$=4) | Ctl-7 ($n$=4) | *P* value |
|---|---|---|---|
| Tumor volume[a] ($mm^3$) | 399±101 | 357±74 | 0.75 |
| Histologically determined largest dimension[a] (mm) | 4.8±0.6 | 5.1±0.4 | 0.61 |
| Tubule formation | | | |
| 1 (>75%) | 0 (7%) | 1 (13%) | |
| 2 (10–75%) | 0 (11%) | 3 (15%) | |
| 3 (<10%) | 4 (31%) | 0 (23%) | **0.03** |
| Invasive lesions | | | |
| Present | 4 (100%) | 0 (0%) | |
| Absent | 0 (0%) | 4 (100%) | **0.01** |
| Ki-67 LI of the tumor[a] (%) | 54.5±4.6 | 49.5±7.1 | 0.58 |

Tumor tissues were resected at 2 months after the injection, and were subsequently fixed in 10% formalin and embedded in paraffin wax. Tumor volume was evaluated by a formula for a semiellipsoid ($4/3\pi r^3/2$). *P* values <0.05 were considered significant and described as boldface.
[a]Data are presented as mean±S.D. All other values represent the number of cases and percentage.

(the relative value of more than 2.0) induced by estradiol in various carcinoma cell lines derived from breast (MCF-7 and MCF-7 c9), endometrium (Ishikawa), ovary (SK-OV-3), and stomach (MKN-28; Inoue *et al.* 2002, 2004, Hayashi *et al.* 2003). Induction of EGR3 mRNA was detected at 6 h after estradiol treatment (10 nM) and reached the maximal level at 24–72 h in MCF-7 cells by northern blot analysis (Inoue *et al.* 2004), and ERE sequence was identified at 2.3 kb from the most upstream mRNA 5′ end of *Egr3* (Bourdeau *et al.* 2004). The biological estrogenic actions are mainly mediated through ERα (Korach 1994, Hayashi *et al.* 2003), and MCF-7 cells highly express ERα but low level of ERβ (Vladusic *et al.* 2000). Therefore, results from our present study suggest that EGR3 is expressed in the breast carcinoma cells, mainly through ERα, as a result of estrogenic action.

We also found that 21 out of 55 cases were immunopositive for EGR3 in breast carcinoma tissues negative for ERα (LI of <10%). This is partly because EGR3 expression was induced by a low or undetectable level of ERα. However, EGR3 was also reported to be induced by various factors, including mitogenic stimulation (Patwardhan *et al.* 1991, O'Donovan *et al.* 1998, Mercier *et al.* 2001, Jouvert *et al.* 2002). Therefore, factors other than estrogen may also be partly involved in the regulation of EGR3 expression in some breast carcinomas.

In our study, EGR3 immunoreactivity was inversely associated with tubule formation, and positively correlated with metastatic lesions of lymph nodes or other organs in the breast carcinomas. Moreover, overexpression of *Egr3* significantly enhanced invasion properties in MCF-7 cells in both *in vitro* study and nude mouse xenograft model. Therefore, EGR3 is postulated to play a pivotal role in carcinoma cell invasion mediated by estrogens in breast carcinomas. Metastasis is the major cause of treatment failure and death of carcinoma patients, and it is a multi-step process that involves not only invasion of carcinoma cells but also lymphogenous and/or hematogenous spread and cell proliferation in the metastatic sites. In our present study, EGR3 immunoreactivity was not associated with tumor size or Ki-67 LI in the breast carcinoma tissues, and overexpression of *Egr3* was not necessarily involved in the cell proliferation or apoptosis status in MCF-7 cells. Therefore, cooperation with EGR3 and other factors may be required for the metastasis of ER-positive breast carcinoma. It awaits further examinations for the detailed clarification of estrogen-mediated metastatic process, because biological function of a great majority of estrogen-responsive genes currently remains unclear. However, for instance, cyclin D (Steeg & Zhou 1998) and estrogen-responsive finger protein (Efp; Urano *et al.* 2002, Suzuki *et al.* 2005*b*) were shown to induce the estrogen-mediated proliferation in breast carcinoma cells, and histone deacetylase (HDAC) 6 were reported as a regulator of cell motility in ER-positive breast carcinoma cells (Saji *et al.* 2005).

Both uni- and multivariate analyses in our study have demonstrated that EGR3 immunoreactivity is a potent prognostic factor for both the recurrence and overall survival in breast carcinoma patients, and similar tendency was also detected in the patients who received tamoxifen therapy. Estradiol is well known to be locally produced and act in breast carcinomas

regardless of the menopausal status (Suzuki *et al.* 2005*a*). In the present *in vitro* experiments, tamoxifen suppressed estradiol-mediated expression of EGR3 mRNA in a dose-dependent manner, but the EGR3 mRNA level in MCF-7 cells treated with estradiol and 10 μM tamoxifen was significantly higher than the control level. Optimal concentrations of tamoxifen were generally considered 10 nM to 10 μM in *in vitro* studies (Vendrell *et al.* 2005), and serum concentration of tamoxifen was reported at 1.8 μM in patients who received high-dose tamoxifen (320 mg), nevertheless 20 mg tamoxifen is usually administrated in breast carcinoma patients. Therefore, tamoxifen may not completely block the estradiol-mediated EGR3 expression in the breast carcinoma patients.

Regarding the molecular mechanism leading to tamoxifen resistance, recent studies demonstrated that breast carcinoma cells adapt by changing their response to estradiol and developing an increased sensitivity to the growth-stimulating action (Martin *et al.* 2003, Berstein *et al.* 2004, Santen *et al.* 2004). These processes are called 'hypersensitivity to estradiol', and the potential association with increased concentrations of ERα and ER-mediated events is proposed (Santen *et al.* 2001, Chan *et al.* 2002, Vendrell *et al.* 2005). In this study, EGR3 mRNA level in LY-2 cells was 5.5-fold higher than that in MCF-7 cells in the absence of exogenous estradiol, but it was dose-dependently decreased by ICI 182 780. In addition, LY-2 cells showed marked amplitude of estradiol-mediated EGR3 mRNA expression when compared with MCF-7 cells. Therefore, it is suggested that EGR3 expression is mainly mediated through ER in LY-2 cells, and these findings of our present study are possibly explained by the hypersensitivity to estradiol in tamoxifen-resistant state of MCF-7 cells. Considering that the EGR3 mRNA level in LY-2 cells treated with estradiol and 10 μM tamoxifen was 2.8-fold higher than that in MCF-7 cells treated with estradiol alone, EGR3 may play an important role also in the tamoxifen-resistant breast carcinoma patients. Therefore, residual carcinoma cells following surgical treatment in EGR3-positive breast carcinomas could rapidly invade in the presence of local estrogens regardless of the tamoxifen therapy, thereby resulting in an increased recurrence and poor prognosis in these patients.

In summary, EGR3 immunoreactivity was detected in carcinoma cells in 52% of breast carcinoma tissues in this study, and it was associated with its mRNA level. EGR3 immunoreactivity was positively associated with lymph node status, distant metastasis into other organs, ERα, or EGR3 immunoreactivity in the recurrent lesions, and negatively correlated with tubule formation. EGR3 immunoreactivity was significantly associated with an increased risk of recurrence or worse prognosis, regardless of the tamoxifen therapy. Estradiol significantly induced EGR3 mRNA expression in a dose-dependent manner in MCF-7 cells, which was markedly amplified in a tamoxifen-resistant MCF-7 cell variant (LY-2). Tamoxifen suppressed the estradiol-meditated induction of EGR3 mRNA in a dose-dependent manner in these cells, but tamoxifen could not inhibit its expression completely. *Egr3*-expressing MCF-7 cells significantly increased the invasion property, but not cell proliferation, both *in vitro* and *in vivo* experiments. These results from our present study suggest that EGR3 plays an important role in estrogen-meditated invasion and is a potent prognostic factor in human breast carcinoma.

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Original Article

# Prognostic Significance of Insulin-like Growth Factor Binding Protein (*IGF*BP)-4 and *IGF*BP-5 Expression in Breast Cancer

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**Background:** Expression of estrogen-regulated genes has been considered as potential predictive markers for endocrine therapy. We focused on two insulin-like growth factor binding proteins (IGFBPs): *IGF*BP-4, which is an early-responsive estrogen-induced gene, and *IGF*BP-5, which is an estrogen-repressed gene. Investigation of *IGF*BP-4 and *IGF*BP-5 expression would provide important information for predicting prognosis and endocrine responsiveness.
**Methods:** The levels of *IGF*BP-4 and *IGF*BP-5 mRNA expression in 162 human breast cancer tissues were analyzed using quantitative real-time reverse transcriptase-PCR. The association between *IGF*BP-4 and *IGF*BP-5 expression and clinicopathological factors was then analyzed.
**Results:** The levels of *IGF*BP-4 and *IGF*BP-5 mRNA expression were positively correlated with estrogen receptor (ER) and progesterone receptor (PgR) status and were negatively correlated with HER2 overexpression. Patients with a high level of *IGF*BP-4 mRNA expression had better disease-free and overall survival than those with a low expression. Multivariate analysis showed that *IGF*BP-4 mRNA expression is an independent prognostic factor for disease-free survival. When analyzed in 116 patients with ER-positive breast cancer, patients whose tumor expressed higher levels of *IGF*BP-4 mRNA or lower levels of *IGF*BP-5 mRNA had better disease-free survival.
**Conclusion:** *IGF*BP-4 mRNA expression was an independent prognostic factor in breast cancer, and patients with ER-positive breast cancer whose tumor expressed higher levels of *IGF*BP-4 and lower levels of *IGF*BP-5 had a better prognosis than those without such findings.

*Key words: breast cancer – IGFBP-4 – IGFBP-5 – prognosis*

## INTRODUCTION

Endocrine therapy has become the most important treatment option for women with estrogen receptor (ER)-positive breast cancer. Nevertheless, many breast cancer patients with tumors expressing high levels of ER are unresponsive to endocrine therapy and all patients with advanced disease eventually develop resistance to the therapy (1). Expression of estrogen-regulated genes has been considered to provide predictive markers for endocrine therapy, because their expression may indicate the presence of a functional estrogen-signaling pathway. Using microarray technology, we have identified more than 100 estrogen-regulated genes in MCF-7 human breast cancer cells (2). Of these

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estrogen-regulated genes, in the present study we focused on two insulin-like growth factor binding proteins (IGFBPs): *IGF*BP-4, which is an early-responsive estrogen-induced gene, and *IGF*BP-5, which is an estrogen-repressed gene.

IGFBPs are considered to bind to insulin-like growth factor (IGF)-I and IGF-II in the extracelluar space, regulating access of IGFs to IGF receptors (3), which is one of the most critical steps for proliferation of breast cancer cells. There are six IGFBPs, *IGF*BP-1 to *IGF*BP-6, which share 40–60% amino acid identity. IGFBPs bind IGF-I and IGF-II with high affinity, and are essential to transport IGFs, to prolong half-lives, and to regulate the availability of free IGFs for interaction with IGF receptors, thereby modulating the effects of IGFs on growth and differentiation. In addition, recent evidence indicates that some IGFBPs may themselves have direct receptor-mediated effects, independent of IGFs (4). *IGF*BP-3 is the most abundant IGFBP in human serum and has been shown to be a growth inhibitory, apoptosis-inducing molecule, capable of acting via IGF-dependent and IGF-independent mechanisms (5). The clinical data presented to date provide ambiguous evidence as to whether the IGFBPs, and in particular *IGF*BP-3, predict a good or poor prognosis in breast cancer (6). Recent studies indicated that high concentrations of IGF-I and *IGF*BP-3 in the circulation were associated with an increased risk of premenopausal breast cancer (7). *IGF*BP-4 appears to be a potent inhibitor of IGF function in several human cell lines (8–10). *IGF*BP-5 plays a critical role in mammary gland development, and, in particular, the removal of mammary epithelial cells by apoptosis that takes place during the involutionary stage of the lactating gland (11). However, little is known about the role of *IGF*BP-4 and *IGF*BP-5 in breast cancer.

In the present study, we examined mRNA and protein expression of *IGF*BP-4 and *IGF*BP-5 in 162 human breast cancer tissues and analyzed their significance for prognosis.

## PATIENTS AND METHODS

### Patients and Tumor Samples

Primary invasive breast carcinoma specimens were obtained by surgical excision from 162 female patients at Nagoya City University Hospital between 1992 and 2000. Informed consent was obtained from all patients before surgery. The study protocol was approved by the institutional review board and conformed with the guidelines of the 1975 Declaration of Helsinki. The median age of the patients was 57.9 years (range, 28–88 years). The patients' tumors were classified with the International Union Against Cancer (UICC) staging system as follows: 44 cases were classified as stage I, 98 cases as stage II, 17 cases as stage III and 3 cases as stage IV. Patients were graded histopathologically according to the modified Bloom and Richardson method proposed by Elston and Ellis (12). As post-operative adjuvant treatment, tamoxifen was given to patients with ER- and/or progesterone receptor (PgR)-positive tumors. Depending on tumor stage, the following chemotherapy regimens were given: oral 5-fluorouracil, CMF, or FEC. Since 1995, post-operative treatment has been done with reference to the recommendation of St Gullen (13). After surgery, 26 patients (16.0%) received no additional therapy. Of the remaining 136 patients, 82 (50.6%) received systemic therapy consisting of endocrine therapy alone, 10 (6.2%) received chemotherapy alone and 44 (27.2%) received combined endocrine therapy and chemotherapy. Patients were observed for disease recurrence and death at least once every 6 months for 5 years after surgery and yearly thereafter. The median follow-up period was 67 months (range, 2–128 months). Samples were snap-frozen in liquid nitrogen and stored at −80°C until RNA extraction.

### Isolation of Total RNA and Reverse Transcription

Total RNA from homogeneous breast cancer tissue, which was microscopically confirmed, was isolated from approximately 500 mg of frozen specimen. Total RNA was also isolated from one flask of HepG2 cells and T47D cells for use as a positive control and to generate standard curves. mRNA was isolated using the TRIZOL reagent (Life Technologies, Inc., Tokyo, Japan) according to the manufacturer's instructions. Reverse transcription reactions were done as previously described (14).

### Primers and Probes

We conducted BLAST searches (Genbank) to confirm the specificity of the nucleotide sequences chosen for the primers and probes and to confirm the absence of DNA polymorphism. To avoid detection of contaminating genomic DNA, the primers for *IGF*BP-4 were located at exon 1 and exon 2, and the primers for *IGF*BP-5 were located at exon 3 and exon 4. The specific oligonucleotide primers were synthesized according to published information as follows: *IGF*BP-4, 5′ sense TCGAGGCCATCCAGGAAA (602–619) and 3′ antisense CCCCATTGACCTTCATCTT (766–748) (165 bp); *IGF*BP-5, 5′ sense CTGTGTACCTGC CCAAT (1411–1427) and 3′ antisense CACTGAAAG TCCCCGTCAA (1561–1543) (151 bp). The donor probe for *IGF*BP-4, 5′-AGCGCCCATGACCGCAG-3′ has a fluorescein label at its 3′ end and the acceptor probe for *IGF*BP-4 5′-TGCCTGCAGAAGCACTTC GC-3′ has LC Red 640 at its 5′ end. For *IGF*BP-5, the donor probe 5′-CCGCAAACGT GGCATCTGCT-3′ and the acceptor probe 5′-GTGCGTGGA CAAGTACGGGATGA-3′ were used.

To ensure the fidelity of mRNA extraction and reverse transcription, all samples were subjected to PCR amplification with oligonucleotide primers and probes specific for the constitutively expressed gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and normalized. *GAPDH* primers were as follows: 5′ sense AAATCAAGTGGGGCGATGCTG and 3′ antisense GCAGAGATGATGACCCTTTTG. The

sequences of the *GAPDH* probes were as follows: the donor probe, 5′-AGAAGGCTGGGGCTCATTTGCAGGG-3′ and the acceptor probe, 5′-GTCCACTGGCGTCTTCACCAC CATG-3′. All primers and probes were purchased from the Japanese Gene Institute (Saitama, Japan).

### Real-time Reverse Transcription-PCR

Real-time reverse transcription-PCR was done using a LightCycler (Roche Molecular Biochemicals, Mannheim, Germany) as previously reported (15). The PCR reaction for *IGF*BP-4 and *IGF*BP-5 was carried out in a final volume of 20 μl containing 2.4 μl of 25 mmol/l $MgCl_2$; 0.5 μl of 20 pmol/μl sense primer and antisense primer; 0.4 μl of 10 pmol/μl donor and acceptor probe; 2 μl of PCR master mix; 1.5 μl of cDNA and made up to 20 μl with water. After an initial denaturation step at 95°C for 60 s, temperature cycling was initiated. Each cycle consisted of denaturation at 95°C for 0 s, hybridization at 57°C for 5 s, and elongation at 72°C for 6 s. The fluorescence signal was acquired at the end of the hybridization step. A total of 55 cycles were performed. Cycling conditions for *GAPDH* were as follows: initial denaturation at 95°C for 60 s, followed by 50 cycles at 95°C for 0 s, 60°C for 5 s and 72°C for 8 s.

### Standard Curves and Presentation of Results

For each PCR run, a standard curve was constructed with serial dilutions of cDNA obtained each from HepG2 cells for *IGF*BP-4 and T47D cells for *IGF*BP-5. The level of expression of *IGF*BP-4 and *IGF*BP-5 mRNA were given as relative copy numbers normalized against *GAPDH* mRNA and shown as mean ± SD. Relative *IGF*BP-4 and *IGF*BP-5 mRNA expression was calculated by the formula: (*IGF*BP-4/*GAPDH*) × 1000 and (*IGF*BP-5/*GAPDH*) × 100, respectively.

A non-template negative control was included in each experiment. All of the non-template negative controls, the standard cDNA dilutions from HepG2 cells or T47D cells, and the tumor samples were assayed in duplicate. All of the patient samples with a coefficient of variation for gene mRNA copy number data >10% were retested using the method of Bieche et al. (16).

### Immunohistochemical Staining of ER and PgR

Immunohistochemical staining of ER and PgR was done using monoclonal mouse antihuman ERα antibody (1D5, DAKO) at 1:100 dilution for ER and monoclonal mouse antihuman PgR antibody (636, DAKO) at 1:100 dilution for PgR as primary antibodies as previously described (17). The expression of ER and PgR was estimated in accordance with the procedure of Allred and colleagues (18). In brief, a proportion score represented the estimated proportion of tumor cells staining positive, as follows: 0 (none); 1 (<1/100); 2 (1/100 to 1/10); 3 (1/10 to 1/3); 4 (1/3 to 2/3); and 5 (>2/3). Any brown nuclear staining in invasive breast epithelium counted towards the proportion score. An intensity score represented the average intensity of the positive cells, as follows: 0 (none); 1 (weak); 2 (intermediate); and 3 (strong). The proportion and intensity scores were then added to obtain a total score, which could range from 0 to 8. Tumors with a score of 3 or greater were considered to be positive for ER or PgR expression.

### Statistical Analysis

Unpaired *t* test was used for the statistical analysis of the association between *IGF*BP-4 and *IGF*BP-5 mRNA expression and clinicopathological factors. Disease-free and overall survival curves were generated by the Kaplan–Meier method and verified with the log-rank test. Cox's proportional hazards model was used for univariate and multivariate analyses of prognostic values. Differences were considered significant when a $P < 0.05$ was obtained.

## RESULTS

### Patient Demographics and Tumor Characteristics

Clinical characteristics are summarized in Table 1. The amount of *IGF*BP-4 mRNA in the tissue samples from 162 patients ranged from 17 to 2561 relative copy numbers (mean, 310.4), whereas the amount of *IGF*BP-5 mRNA ranged from 3 to 4060 relative copy numbers (mean, 165.6).

**Table 1.** Adjuvant systemic treatments for patients after surgery

| Adjuvant therapy | No. (%) |
|---|---|
| Total patients | 162 |
| None | 26 (16.0) |
| Endocrine therapy | 82 (50.6) |
| Tamoxifen | 72 |
| LHRH agonist | 2 |
| LHRH agonist + tamoxifen | 7 |
| Aromatase inhibitors | 1 |
| Chemotherapy | 10 (6.2) |
| Oral 5-fluorouracil | 6 |
| CMF | 4 |
| Combined | 44 (27.2) |
| Tamoxifen + oral 5-fluorouracil | 37 |
| Tamoxifen + CMF | 1 |
| Tamoxifen + CAF | 2 |
| LHRH agonist + tamoxifen + CMF | 3 |
| LHRH agonist + tamoxifen + paclitaxel | 1 |

LHRH, luteinising hormone-releasing hormone;
CMF, cyclophosphamide methotrexate 5-fluorouracil;
CAF, cyclophosphamide adriamycin 5-fluorouracil.

### Correlation between *IGF*BP-4 and *IGF*BP-5 mRNA Expression and Clinicopathological Factors

The level of *IGF*BP-4 mRNA expression was significantly correlated with histological grade ($P = 0.0032$). Positive associations were observed between *IGF*BP-4 mRNA expression and ER ($P = 0.0031$) and PgR ($P = 0.0045$) expression. An inverse correlation was found between *IGF*BP-4 mRNA expression and HER2 overexpression ($P = 0.0007$). No association was found between *IGF*BP-5 mRNA expression and histological grade, ER, PgR and HER2 expression (Table 2).

There was no association between *IGF*BP-4 and *IGF*BP-5 mRNA expression and age, menopausal status, tumor size, or lymph node status. Interestingly, *IGF*BP-4 mRNA expression was strongly correlated with *IGF*BP-5 mRNA expression (Fig. 1).

### Patients whose Tumor Expressed Higher Levels of *IGF*BP-4 mRNA had Better Disease-free and Overall Survival

To identify a clinically meaningful cutoff point for levels of *IGF*BP-4 and *IGF*BP-5 mRNA expression that could be used in disease prognosis analysis, various levels of *IGF*BP-4 and *IGF*BP-5 mRNA expression were tested using the Kaplan–Meier method and verified by the log-rank test. When analyzing disease-free and overall survival, the cutoff points for the levels of *IGF*BP-4 and *IGF*BP-5 mRNA were set at 205 and 38, respectively. Patients with a high level of *IGF*BP-4 mRNA expression ($470.2 \pm 366.2$; $n = 86$) had better disease-free survival than those with a low expression ($102.9 \pm 54.0$; $n = 72$) ($P = 0.0002$, Fig. 2a). Similarly, patients with a high level of *IGF*BP-4 mRNA expression had better overall survival than those with a low level of expression ($P = 0.022$, Fig. 2b). However, *IGF*BP-5 mRNA expression status did not affect disease-free or overall survival (Fig. 2c and d).

### *IGF*BP-4 mRNA Expression is an Independent Prognostic Factor of Disease-free Survival in Breast Cancer

Univariate analysis demonstrated that *IGF*BP-4 mRNA expression ($P = 0.0044$), as well as tumor size ($P = 0.016$), lymph node status ($P < 0.0001$), ER ($P = 0.0016$), PgR ($P = 0.031$), HER2 ($P = 0.031$), and the type of adjuvant therapy ($P = 0.028$) was strongly able to predict disease-free survival (Table 3). In multivariate analysis, patients with tumors with high *IGF*BP-4 mRNA expression ($P = 0.049$), negative lymph node status ($P = 0.012$), and the type of adjuvant therapy ($P = 0.031$) had significantly increased disease-free survival (Table 3). For overall survival, univariate analysis (Table 4) showed significant associations between overall survival and *IGF*BP-4 mRNA expression ($P = 0.027$), lymph node status ($P = 0.0001$), histological grade ($P = 0.010$), ER ($P = 0.0002$), PgR ($P = 0.0027$) and HER2 ($P = 0.023$). There was no significant relation between overall survival and *IGF*BP-4 mRNA expression in multivariate analysis (Table 4). We concluded from these

**Table 2.** Correlation between clinicopathological factors and *IGF*BP-4 and *IGF*BP-5 of 162 breast cancer patients

| | | No. of patients | IGFBP-4 mRNA | *P* | IGFBP-5 mRNA | *P* |
|---|---|---|---|---|---|---|
| Age (years) | 50 | 55 | 285.3 ± 376.9 | 0.48 | 97.5 ± 123.0 | 0.13 |
| | >50 | 107 | 323.6 ± 299.2 | | 200.5 ± 496.4 | |
| Menopausal status | Pre | 67 | 281.2 ± 346.7 | 0.34 | 135.8 ± 354.9 | 0.43 |
| | Post | 95 | 331.6 ± 312.9 | | 187.2 ± 449.4 | |
| Tumor size (cm) | <2.0 | 31 | 362.4 ± 322.8 | 0.12 | 133.3 ± 148.1 | 0.67 |
| | 2.0 | 126 | 275.5 ± 256.7 | | 168.9 ± 458.3 | |
| Lymph node status | negative | 90 | 347.4 ± 398.2 | 0.15 | 110.7 ± 160.9 | 0.07 |
| | positive | 61 | 265.0 ± 212.9 | | 195.3 ± 395.5 | |
| Histological grade | 1,2 | 114 | 317.7 ± 280.1 | 0.0032* | 188.2 ± 482.2 | 0.22 |
| | 3 | 36 | 165.0 ± 197.5 | | 87.5 ± 91.4 | |
| ER | negative | 37 | 174.0 ± 222.3 | 0.0031* | 78.8 ± 123.0 | 0.17 |
| | positive | 119 | 323.5 ± 271.5 | | 187.4 ± 469.8 | |
| PgR | negative | 48 | 195.3 ± 245.4 | 0.0045* | 90.0 ± 146.2 | 0.15 |
| | positive | 108 | 328.4 ± 268.0 | | 193.5 ± 488.8 | |
| HER2 | negative | 119 | 327.3 ± 283.3 | 0.0007* | 192.0 ± 472.2 | 0.10 |
| | positive | 34 | 149.6 ± 151.7 | | 59.0 ± 61.7 | |

IGFBP, insulin-like growth factor binding proteins; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor type 2.
*P*, unpaired *t*-test.
*$P < 0.05$.

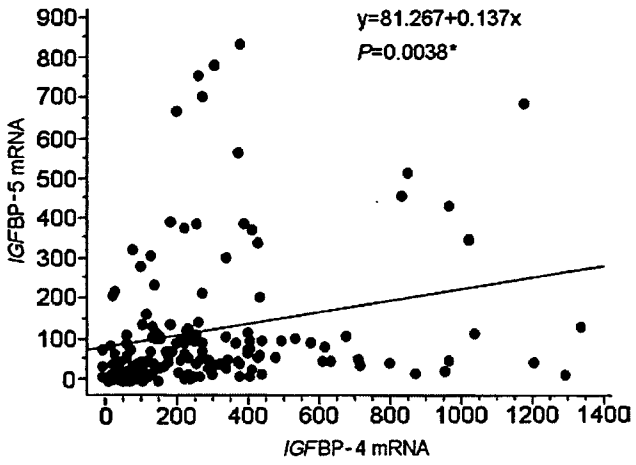


**Figure 1.** Correlation between *IGF*BP-4 and *IGF*BP-5 mRNA expression in human breast carcinomas. Expression is shown as relative copy numbers normalized against *GAPDH* mRNA. *IGF*BP-4, insulin-like growth factor binding protein-4; *IGF*BP-5, insulin-like growth factor binding protein-5.

analyses that *IGF*BP-4 mRNA expression is an independent prognostic factor of disease-free survival in breast cancer.

### Patients with ER-positive Breast Cancer whose Tumor Expressed Higher Levels of *IGF*BP-4 mRNA or Lower Levels of *IGF*BP-5 mRNA had Better Disease-free Survival

We then analyzed disease-free and overall survival in 119 patients with ER-positive breast cancer. Kaplan–Meier analysis of disease-free survival showed that a high level of *IGF*BP-4 mRNA expression (449.1 ± 272.9; $n = 72$) was significantly associated with a reduced risk of recurrence than a low level of *IGF*BP-4 mRNA expression (118.3 ± 52.7; $n = 43$) ($P = 0.018$, Fig. 3a); however, there was no correlation between *IGF*BP-4 mRNA expression and overall survival in these patients (Fig. 3b). Furthermore, only one patient with a tumor that expressed a low level of *IGF*BP-5 mRNA (16.9 ± 9.8; $n = 28$) relapsed ($P = 0.046$, Fig. 3c) and all patients were alive during the follow-up periods (Fig. 3d), whereas 19 patients relapsed who had tumors that expressed a high level of *IGF*BP-5 mRNA (239.9 ± 526.9;

**Table 3.** Prognostic factors in 158 breast cancers compared with disease-free survival

| | Univariate | Multivariate | | |
|---|---|---|---|---|
| | *P* | *P* | Relative risk | 95% confidence interval |
| Age | 0.27 | – | – | – |
| Menopausal status | 0.68 | – | – | – |
| Tumor size | 0.016* | 0.36 | 0.665 | 0.280–1.579 |
| Lymph node status | <0.0001* | 0.012* | 0.419 | 0.213–0.824 |
| Histological grade | 0.087 | – | – | – |
| ER | 0.0016* | 0.052 | 2.369 | 0.991–5.663 |
| PgR | 0.031* | 0.42 | 0.699 | 0.292–1.675 |
| HER2 | 0.031* | 0.47 | 0.764 | 0.367–1.591 |
| IGFBP-4 mRNA | 0.0044* | 0.049* | 0.480 | 0.232–0.996 |
| IGFBP-5 mRNA | 0.50 | – | – | – |
| Adjuvant therapy | 0.028* | 0.031* | 0.111 | 0.015–0.817 |

*$P < 0.05$.

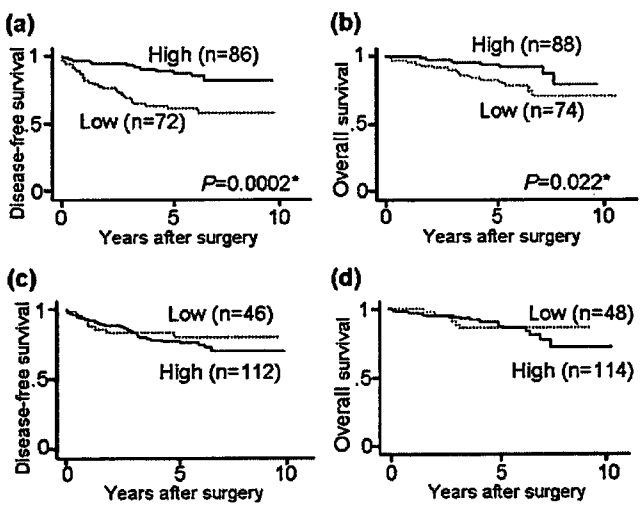


**Figure 2.** Kaplan–Meier analysis of breast cancer patients. Effect of *IGF*BP-4 mRNA expression on disease-free (a) and overall (b) survival among 162 patients with invasive carcinoma and effect of *IGF*BP-5 mRNA expression on disease-free (c) and overall (d) survival among 162 patients with invasive carcinoma.

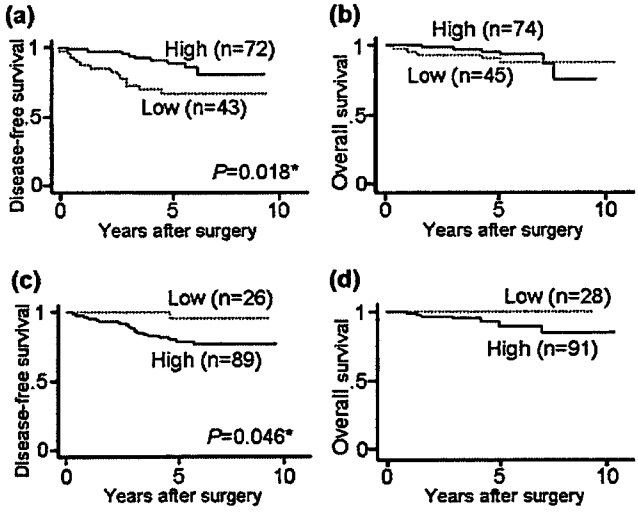


**Figure 3.** Kaplan–Meier analysis of ER-positive breast cancer patients. Effect of *IGF*BP-4 mRNA expression on disease-free (a) and overall (b) survival among 119 patients with ER-positive breast cancer and effect of *IGF*BP-5 mRNA expression on disease-free (c) and overall (d) survival among 119 patients with ER-positive breast cancer.

$n = 89$). Univariate analysis (Table 5) demonstrated that *IGF*BP-4 mRNA expression ($P = 0.022$) as well as lymph node status ($P = 0.0001$) was strongly able to predict risk of recurrence in ER-positive breast cancer. In multivariate analysis (Table 5), patients with tumors with high *IGF*BP-4 mRNA expression had a significantly increased disease-free survival ($P = 0.029$), indicating that *IGF*BP-4 mRNA expression is an independent prognostic factor of disease-free survival in ER-positive breast cancer. There was no significant relation between overall survival and *IGF*BP-4 and *IGF*BP-5 mRNA expression in ER-positive breast cancer (Table 6).

## DISCUSSION

In the present study, we examined mRNA expression of *IGF*BP-4 and *IGF*BP-5 in 162 human breast cancer tissues, and demonstrated that *IGF*BP-4 mRNA expression was an independent prognostic factor in breast cancer, and that patients with ER-positive breast cancer whose tumor expressed higher levels of *IGF*BP-4 mRNA and lower levels of *IGF*BP-5 mRNA had a better prognosis that those without such findings.

Although the roles of *IGF*BP-4 and *IGF*BP-5 in breast cancer are not well established, it is well known that the pattern of *IGF*BP expression and secretion relates to the ER status of breast cancer cells (19). It was also reported that *IGF*BP-4 and *IGF*BP-5 mRNA concentrations were greater in ER-positive cancer tissues than in ER-negative tumors (19,20), and *IGF*BP-4 and *IGF*BP-5 protein expression was correlated positively with ER and PgR (21). Our results also showed that *IGF*BP-4 mRNA expression was positively associated with ER and PgR expression. On the contrary, it was reported that *IGF*BP-3 mRNA and protein levels were found to be inversely correlated with ER and PgR levels (22,23).

Although the role of *IGF*BP-4 in the mammary gland and breast cancer has not been fully elucidated, *IGF*BP-4 has been reported by several laboratories as one of the early-responsive estrogen-induced genes through studies using microarray technology (2,24,25). It was also reported

**Table 4.** Prognostic factors in 162 breast cancers compared with overall survival

| | Univariate | Multivariate | | |
|---|---|---|---|---|
| | *P* | *P* | Relative risk | 95% confidence interval |
| Age | 0.25 | – | – | – |
| Menopausal status | 0.82 | – | – | – |
| Tumor size | 0.089 | – | – | – |
| Lymph node status | 0.0001* | 0.0019* | 0.19 | 0.066–0.54 |
| Histological grade | 0.010* | 0.99 | 1.01 | 0.37–2.75 |
| ER | 0.0002* | 0.091 | 3.39 | 0.82–14.00 |
| PgR | 0.0027* | 0.98 | 1.02 | 0.26–4.05 |
| HER2 | 0.023* | 0.85 | 0.91 | 0.34–2.45 |
| IGFBP-4 mRNA | 0.027* | 0.56 | 1.37 | 0.47–3.87 |
| IGFBP-5 mRNA | 0.24 | – | – | – |
| Adjuvant therapy | 0.11 | | | |

*$P < 0.05$.

**Table 5.** Prognostic factors in 115 ER-positive breast cancers compared with disease-free survival

| | Univariate | Multivariate | | |
|---|---|---|---|---|
| | *P* | *P* | Relative risk | 95% confidence interval |
| Age | 0.92 | – | – | – |
| Menopausal status | 0.99 | – | – | – |
| Tumor size | 0.054 | – | – | – |
| Lymph node status | 0.0001* | <0.0001* | 0.15 | 0.061–0.39 |
| Histological grade | 0.49 | – | – | – |
| PgR | 0.90 | – | – | – |
| HER2 | 0.55 | – | – | – |
| IGFBP-4 mRNA | 0.022* | 0.029* | 0.380 | 0.159–0.906 |
| IGFBP-5 mRNA | 0.08 | – | – | – |
| Adjuvant therapy | 0.16 | | | |

*$P < 0.05$.

**Table 6.** Prognostic factors in 119 ER-positive breast cancers compared with overall survival

| | Univariate | Multivariate | | |
|---|---|---|---|---|
| | *P* | *P* | Relative risk | 95% confidence interval |
| Age | 0.46 | – | – | – |
| Menopausal status | 0.21 | – | – | – |
| Tumor size | 0.17 | – | – | – |
| Lymph node status | 0.020* | 0.036* | 0.18 | 0.038–0.90 |
| Histological grade | 0.63 | – | – | – |
| PgR | 0.52 | – | – | – |
| HER2 | 0.59 | – | – | – |
| IGFBP-4 mRNA | 0.68 | – | – | – |
| IGFBP-5 mRNA | – | – | – | – |
| Adjuvant therapy | 0.68 | | | |

*$P < 0.05$.

that *IGF*BP-4 was up-regulated by estradiol on which ICI182780 acted as an antagonist, whereas tamoxifen and raloxifen acted as partial antagonists (26). We previously reported that expression of histone deacetylase (*HDAC*) 6, which is a late responsive estrogen-induced gene, is correlated with a better prognosis in breast cancer and that expression of higher levels of *HDAC6* tended to be predictive for response to endocrine therapy (14). Our present study showed that *IGF*BP-4 mRNA expression was an independent prognostic factor in breast cancer. Because the number of patients available for evaluating responsiveness to endocrine therapy in this study was limited, further study is needed to analyze whether *IGF*BP-4 is a predictive factor for endocrine therapy. Furthermore, a recent study showed that *IGF*BP-4 is one of the key genes to correlate with tamoxifen resistance by gene expression array and immunohistochemistry tissue micro arrays (27). Because 96 of the 119 patients with ER-positive breast cancer received tamoxifen as an adjuvant therapy in our present study, *IGF*BP-4 expression levels might have affected the tamoxifen response.

However, *IGF*BP-5 is an estrogen-repressed gene and our results indicated that only one patient with a tumor that expressed a low level of *IGF*BP-5 mRNA relapsed and all such patients were alive during the follow-up periods in ER-positive breast cancer. Patients with hormone receptor-positive tumors were given tamoxifen as adjuvant therapy and received endocrine therapy as initial treatment after relapse. Therefore, *IGF*BP-5 expression might be predictive of response to endocrine therapy. Furthermore, studies using a gene expression profile demonstrated that *IGF*BP-5 was a gene signature of a poor prognosis (28), and that *IGF*BP-5 protein expression was elevated in samples of lymph node metastasis (29). Although there was no difference between *IGF*BP-5 expression and lymph node status or survival in any of the patients in our study, *IGF*BP-5 expression might be a poor prognostic factor in breast cancer.

We cannot readily explain why *IGF*BP-4 and *IGF*BP-5 mRNA expressions were shown to be strongly and positively correlated in our study, although higher levels of *IGF*BP-4 and lower levels of *IGF*BP-5 had a better prognosis. Our preliminary immunohistochemical study for *IGF*BP-4 and *IGF*BP-5 protein expression of human breast cancer tissues showed that these proteins were present both in the cytoplasm and the nuclei. Moreover, some *IGF*BP-4 or *IGF*BP-5-positive cells were noted in the stroma of normal breast and carcinoma tissues, and the *IGF*BP-4 or *IGF*BP-5-positive cells in the stroma were considered lymphocytes or macrophages. Further studies are needed to clarify the function of *IGF*BP-4 and *IGF*BP-5 in the cytoplasm and the nuclei of cancer cells and also of stromal cells in order to understand the role of *IGF*BP-4 and *IGF*BP-5 in breast cancer.

The present study demonstrated that *IGF*BP-4 mRNA expression was an independent prognostic factor in breast cancer, and that patients with ER-positive breast cancer whose tumor expressed higher levels of *IGF*BP-4 mRNA and lower levels of *IGF*BP-5 mRNA had a better prognosis that those without such findings.

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**Conflict of interest statement**

None declared.

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# ホルモン療法奏効メカニズムと効果予測
## ——エストロゲンシグナルを標的に
Mechanisms and prediction of hormonal therapy

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◎現在の乳癌のホルモン療法の標的はエストロゲンシグナルである．エストロゲンに依存して生存し，増殖・進展する乳癌細胞に対してはきわめて効果的であり，その作用メカニズムは乳癌の発生・増殖機構と直結した問題として，基礎研究としても重要なテーマである．しかも，その成果は診断と治療に直接役立つことが期待できる．第三世代のアロマターゼ阻害剤の実用化など，近年著しい進歩を遂げると同時に複雑化しているホルモン療法の奏効性予測は，今後の乳癌の個別化治療にとって重要な問題である．遺伝子プロファイル解析などあらたな技術を取り入れた試みが行われており，今後の展開が注目される．

Key word：内分泌療法，エストロゲン，エストロゲン受容体，アロマターゼ

実地臨床で広範に施行されている乳癌のホルモン療法の有用性についてはあらためて述べるまでもないが，近年のLH-RHアゴニストや第三世代のアロマターゼ阻害剤などの登場は，ホルモン療法の有用性をいっそう高めるとともに，術前ホルモン療法のようなあらたな展開をももたらしている．一方，エストロゲンシグナルに関する基礎研究の進展とともに，ホルモン療法の奏効メカニズムについての理解も進んできた．しかし，奏効群と不応群の判別や耐性獲得の機構など臨床的にも重要な課題について，いまだ十分解明されているとはいえない．ホルモン療法の選択肢は多彩になってきており，それらの的確な選択・適用のためにはより高精度な効果予測が求められている．それにより，個々の乳癌患者の特性に合わせた個別化治療が可能になり，ひいては医療コスト削減にも寄与するであろう．

## ホルモン療法の理論的背景

乳癌におけるホルモン療法の基本的な考え方は，エストロゲン依存性に増殖進展する乳癌細胞に対してエストロゲンの供給を遮断する，またはその作用の仲介者である受容体の機能を阻害するなどの手段により，癌細胞の増殖を止め，さらにアポトーシスを誘導するというものである．そのため，古くは閉経前の患者に対しては主要なエストロゲン産生臓器である卵巣を摘除するなどの手段がとられてきた．現在ではLH-RHアゴニスト剤を用いて卵巣機能をブロックしている．また，閉経後の乳癌患者に対しては抗エストロゲン剤，とくに以前からタモキシフェンが使われてきたが，近年，アロマターゼ阻害剤によって局所のエストロゲン合成を阻害することによって，同等，あるいはこれまで以上の効果が得られるようになってきた．

いずれにせよ，乳癌におけるホルモン療法はエストロゲンシグナルの遮断が基本戦略であり，そ

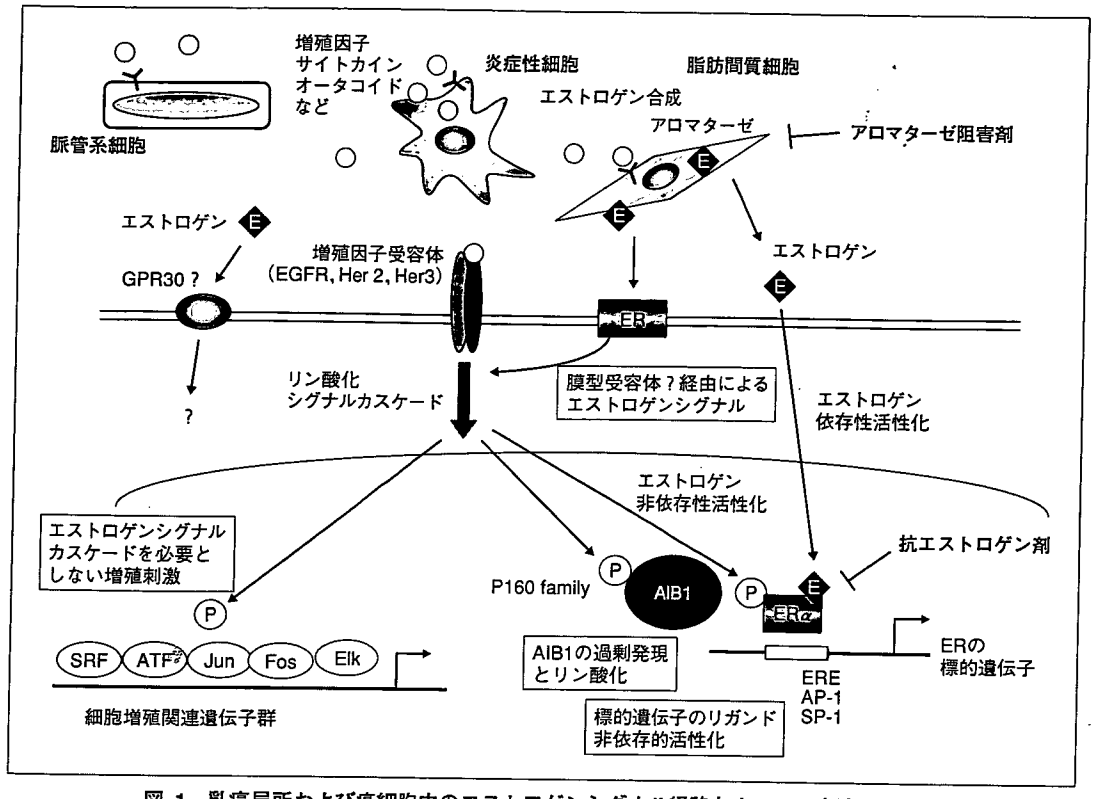


図 1. 乳癌局所および癌細胞内のエストロゲンシグナル経路とホルモン療法の作用点

れが奏効するかどうかは，乳癌細胞がエストロゲン依存性に増殖する性質を保持しているかどうかに強く依存すると思われる．

## ホルモン療法に関連する基礎研究分野の新しい展開

これまでに核内転写因子としてのエストロゲン受容体(ER)の研究は，他の核内受容体研究と連動して急速に進んできた．とくに転写共役因子に関する研究の急速な進展が象徴的である．これによって抗エストロゲン剤の作用機序の分子レベルでの理解が進み，新規 SERM の開発などに大きく寄与してきた．ところが，エストロゲンの多彩な作用は従来の機序だけでは説明できず，近年，細胞質あるいは細胞膜に associate して存在する膜型 ER の存在やそれと関連したリン酸化シグナル経路の重要性が提唱され，それによってタモキシフェン耐性獲得など，臨床上観察される従来の古典的経路では理解が困難な各種の現象を説明する理論的根拠として議論されている(図 1)．またごく最近，従来の経路とはまったく異なった G-coupled protein を介したエストロゲン作用の経路の存在も報告されている．

また，治療の主役が抗エストロゲン剤からアロマターゼ阻害剤などのエストロゲンの供給の遮断に移ってきたことから，乳癌局所の微小環境や癌細胞と周辺間質細胞との機能的相互作用の機構解明の重要性が高まってきた．癌細胞の増殖をサポートする CAF(carcinoma-associated fibroblast)の存在はその象徴である．著者らも乳癌の個々の間質細胞による癌細胞のエストロゲンシグナルの活性化を解析することにより，乳癌微小環境には患者ごとの個性があり，共在する癌細胞の性質と密接に関連していることを明らかにした．

これらの基礎研究の今後の展開は，ホルモン療法の臨床上の問題点の理解とその克服にきわめて重要と思われる．とくに分子標的治療の新しい標的の発見につながる可能性も期待できる．

## ホルモン療法効果予測因子と問題点

2005年のSt. Gallen国際会議ではリスクカテゴリーが大きく変更され[1]，腫瘍径，grade，年齢，リンパ節転移のほかに，あらたに脈間侵襲，HER2発現などの因子が追加された．一方，ERとプロゲステロン受容体(PgR)は，ホルモン感受性，非感受性の分類の指標に用いられ，予測因子として重要視されている．乳癌におけるホルモンレセプターの発現の評価は現在では免疫染色法で行われており，とくに陽性細胞の占有率と染色強度を合わせて評価するAllredスコア分類[2]，あるいはそれに準じた方法が用いられている．しかし，どの数値を閾値としてホルモン療法適応群とするかについては国際的に統一されていない．とくに染色強度に関しては，各施設の手技・手法による違いが問題となるため，客観的評価が難しい．

ホルモン療法の奏効性が前述のように癌細胞のエストロゲン依存性増殖能の有無に強く関連していると考えられることから，治療の対象がER陽性乳癌であることは理にかなっている．しかし，ERの発現があればエストロゲン依存性であるとは限らず，その場合，ERの発現と治療効果が一致しないことが予想され，ER陽性/PgR陰性のようなケースもその一例と思われる．また少数例ではあるが，ER陰性でもホルモン療法が奏効する場合があることも知られており，ホルモンレセプターの存在の有無だけを奏効性の判断基準とするのは万全でない．ERが蛋白として発現し，存在していても何らかの理由でその下流のシグナルカスケードが正常に機能していない場合には，たとえER陽性と判定されてもホルモン療法が奏効しないことは十分考えられる．たとえば，ERを含む転写複合体の活性化がリガンド非依存的に起こっている場合(転写共役因子の過剰発現，リン酸化による恒常的活性化，ER自身のリン酸化による活性化など)，あるいはERは存在していても細胞の増殖がER関連シグナルカスケードに非依存性になっている場合などである．

それぞれのケースで各ホルモン療法の効果にもいろいろなパターンが予想され，それは患者ごとに異なると予想される．抗エストロゲン剤の適応がよいのか，アロマターゼ阻害剤の適応がよいのか，あるいは具体的にどの薬剤がよいのか，患者ごとの正確な予測が可能なあらたな予測因子が求められている．

## ホルモン療法効果予測の基礎研究

一方，より正確に，かつ普遍的に奏効性が予測できるようなあらたな手法・因子を模索する基礎研究も進められている．分子レベルの予後因子としてはこれまでもp53やBcl-2，Ki67，Her2などが検討されてきた．最近ではこのような既知の予後因子も含めた21遺伝子の発現をPCRによって判定する方法(Oncotype DX™)によって効果的な化学療法の適応群を推定しようという試みが行われている[3]．また，マイクロアレイ解析研究から同定した乳癌の予後予測に有用な70遺伝子のサブセットを解析することによって，実用的な予後予測をめざす研究(MINDACT Trial)もある．

しかし，これらはホルモン療法の効果予測をめざしたものではない．これまで，pS2やカテプシンDなどの以前から知られているER標的遺伝子について，ER，PgR以上の効果的な予測因子になりうるかの検討が行われてきたが，期待される結果は得られていない．ERの標的遺伝子についてはこれまで培養細胞を用いた研究が中心であり，結果が特定の細胞株においてのみ観察されるものであることが一因かもしれない．

ひとつの手段として考えられる戦略は，乳癌で発現が変動しているエストロゲン標的遺伝子を網羅的にとらえて，そのプロファイルを解析することにより，患者ごとのエストロゲンシグナル経路がどのような状況にあるのか把握し，最適な治療法を見出す，という手法である．すなわち，乳癌のホルモン療法の作用機序がエストロゲン経路の遮断であるなら，その反応性は標的遺伝子群のプロファイルとしてとらえることができるのではないかという考え方である．著者らは以前からこのような方法でホルモン療法の治療効果予測をめざした研究を行っているが，詳細は原著[4,5]や他の総説[6,7]を参考にしていただきたい．

研究が進んで奏効性予測が可能な新規候補遺伝子の数が絞り込まれれば，従来の免疫染色法，またはマルチプレックスRT-PCR法など，より簡便

で実用的な手法による奏効性予測が可能かもしれない．これまでの研究の過程で他施設との共同研究によってHDAC6[8,9]やIGFBP4，EGR3[10]などのエストロゲン応答遺伝子で，臨床病理学的因子や予後などに関連する新規因子を同定してきた．これらはそれぞれ単独ではER/PgRを超えるような強力な予測因子ではないが，複数遺伝子による診断用コンテンツとしては有用であろう．さらに，今後の研究の進展とともにあらたな因子が見出される可能性も考えられる．

## アロマターゼ阻害剤の奏効性予測

アロマターゼ阻害剤の作用機序は，局所でのアロマターゼ酵素の活性を阻害して乳癌細胞へのエストロゲンの供給を遮断することである(図1)．癌細胞にもアロマターゼがあるという報告もあるが，大部分は腫瘍周辺の間質細胞に存在し，パラクリンにエストロゲンを供給している．ならば個々の乳癌に存在する間質の個性も治療効果に大きく影響するはずである．しかも癌細胞は，エストロゲンだけでなく他の増殖因子など，癌微小環境に存在するさまざまな因子によりその生存が支えられている．著者らは実際に，ERを活性化する間質細胞の能力は症例ごとに異なっていることを明らかにした[11]．高精度な治療奏効性予測には，これらの癌微小環境の特徴・個性を考慮することは必須であろう．そこで，エストロゲンシグナル活性を反映して蛍光を発する細胞やウイルスベクターを使用し，個々の乳癌の微小環境を解析する，あるいはアロマターゼ阻害剤の効果を *in vitro* で評価することを可能とする新しい解析法の開発を進めている．

## 今後のホルモン療法奏効性予測の基礎研究の展望

ホルモン療法は乳癌のホルモン依存性という生物学的特性に依存した治療法であるため，その治療効果は個々の腫瘍の生物学的個性に依存する．その個性の把握に個々の腫瘍のエストロゲンシグナル状態を知ることは重要であり，治療奏効性予測のためには必須と思われる．しかし，現時点では乳癌の場合に限らず，多数遺伝子の発現プロファイル解析を診断に応用しようという試みは，当初期待されていたほどのめざしい成果をあげていない．技術的問題点や標準化など，多くの困難が臨床への応用を難しくしている現実があるが，前述のような試みも含め，その克服のための研究は徐々に進んでおり，大きな流れは変わらないと思われる．

今後このような，ある程度まとまった数の遺伝子の発現をプロファイルという形でとらえ，スコア化して予後予測や感受性予測の指標とするという手法が普及してくる可能性は十分ある．一方，その実現のためにも乳癌の発生と増殖・進展のメカニズムに関する基礎研究のよりいっそうの進展は不可欠である．基礎研究と臨床研究が両輪となって今後の新規感受性予測因子の研究と先端技術に基づく診断技術が進歩し，患者にとってよりよい乳癌個別化治療が可能となっていくことを期待したい．

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# 乳　癌

## —基礎・臨床研究のアップデート—

基礎研究

VI. 治療に向けての基礎研究

## 内分泌療法感受性予測因子

林　慎一

# 内分泌療法感受性予測因子

Predictive factors for endocrine therapy

林　慎一

Key words：ホルモン療法，エストロゲン，SERM，抗エストロゲン剤，アロマターゼ阻害剤

## はじめに

乳癌の薬物治療の中で内分泌療法は極めて重要な位置を占めており，あらゆる局面で広範に施行されている．なかでも術後内分泌療法はエストロゲン受容体(ER)陽性患者の再発を約半分に低減させるという著効を示し，しかもその有害事象は化学療法に比較するとかなり少ない．このような内分泌治療が効果的に広く行われていること自体が，他の癌にはみられない乳癌の治療の特徴ともいえよう．

一方，治療開始前に感受性群，非感受性群を正確に層別化する予測因子は現時点では見いだされていない．言うまでもなく，患者個々の治療効果を正確に予測し，適切な治療法を選択することは極めて重要である．特に近年，内分泌療法はLH-RHアゴニスト剤や複数の第3世代のアロマターゼ阻害剤の登場によって，急速に進歩しており，それに伴って治療の選択肢は多彩になってきており，それらの的確な選択，適応のためには，より精度の高い新しい効果予測因子が求められている．高精度な内分泌療法の効果予測は個々の乳癌患者の特性に合わせた個別化治療を可能にし，ひいては医療コストの低減にも貢献するであろう．

## 1. 乳癌に対する内分泌療法の概念

乳癌における内分泌療法の基本的な戦略は，エストロゲン依存性に増殖進展する乳癌細胞に対して，ERをブロックする，ないしはエストロゲンそのものの供給を遮断してしまうなどの手段により，その増殖を止めてしまう，あるいはアポトーシスを誘導するというものである．そのため，古くは閉経前の患者に対しては主要なエストロゲン産生臓器である卵巣を摘除するなどの手段がとられてきた．現在ではLH-RHアゴニスト剤を用いて卵巣に対するエストロゲン産生刺激をブロックすることによって同様の結果が得られるようになった．また，閉経後の乳癌患者に対しては抗エストロゲン剤，特にタモキシフェンが使われてきた．近年ではアロマターゼ阻害剤によって局所のエストロゲン合成を阻害することによって同等の，あるいは抗エストロゲン剤以上の効果が得られるようになってきた．これらの詳細は他稿を参照されたい．

いずれにせよ，乳癌における内分泌治療はエストロゲンシグナルのブロックが基本戦略であり，その戦略が奏効するか否かは乳癌細胞がエストロゲン依存性に増殖する性質を保持しているかどうかに強く依存する．よって内分泌療法の奏効性は乳癌細胞のエストロゲン依存性の有無，あるいはその強弱にかかっているといえる．

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## 2. 術後内分泌療法感受性予測因子

乳癌の内分泌治療で最も多くの患者に施行されているのが術後の補助療法である．実地臨床で行われる乳癌の術後治療に関しては，St. Gallen国際会議のような乳癌の初期治療の国際的合意を目的とした会議の結果が大きく影響する．2005年の会議ではリスクカテゴリーが大きく変更され[1]，腫瘍径，grade，年齢，リンパ節転移のほかに新たに脈管侵襲，HER2発現などの因子が追加された．一方，ERとプロゲステロン受容体(PgR)はリスクから外され，ホルモン感受性，非感受性の分類に用いられている．その点からもER/PgRは予測因子として重要視されていることがわかる．

乳癌におけるホルモン受容体の発現の評価は，以前はEIA法が標準的に用いられてきたが，現在は免疫染色法で行われており，特に陽性細胞の占有率と染色強度を合わせて評価するAllredスコア分類[2]，もしくはそれに準じた方法が用いられている．しかし，どの数値を閾値として内分泌療法適応群とするのが臨床上最適であるかについてはいろいろな考え方があり，国際的に統一されているわけではない．特に染色強度に関しては各施設の手技，手法による違いが問題となるため評価が難しい．

一方，より正確に，かつ普遍的に奏効性が予測できるような新たな手法，因子を模索する基礎研究も進められている．分子レベルの因子としては，これまでもp53やBcl-2，Ki67，HER2などがレトロスペクティブに再発や生存率との関係から検討されてきた．しかし，その多くはいわゆる予後因子であって，内分泌療法の予測因子ではない．ただし，HER2に関してはトラスツズマブを用いる治療の効果予測因子といえる．また，pS2やカテプシンDなどの以前からよく知られているER標的遺伝子についても検討が行われてきたが，確立した評価は得られていない．ERの標的遺伝子についてはこれまで，培養細胞を用いた研究が中心であり，結果が特定の細胞株においてのみ観察されるものであることも多いと思われる(MCF-7でのみ誘導がみられるpS2が良い例である)．

## 3. ER/PgR発現を予測因子とする問題点

内分泌療法の奏効性が癌細胞のエストロゲン依存性の有無に強く関連していると考えられることから，内分泌治療の対象がエストロゲン受容体を発現しているER陽性乳癌であることは理にかなっていると思われる．実際，過去においても，また現時点でも最も臨床の現場で汎用され，信頼性の高い分子レベルの予測因子はERの発現である．更にERの標的遺伝子の一つであるPgRも補助的に用いられている．

しかし，ERの発現があることがそのままエストロゲン依存性をもつということと同義でないことも事実であり，その点がERの発現と治療効果が一致しないことがあることやER陽性/PgR陰性のようなケースを生じる原因となっていると思われる．すなわち，ホルモン受容体の存在の有無だけを奏効性の判断基準とするのは万全でない．ERが蛋白として発現し，存在していても何らかの理由でその下流のシグナルカスケードが正常に機能していない場合などには，たとえER陽性と判定されても内分泌療法が奏効しないことは十分考えられる．

その場合，注意しなければならないことは，エストロゲンシグナルカスケードが正常に機能していないというケースも複数のパターンが考えられるという点である(**図1**)．例えばERを含む転写複合体の活性化がリガンド非依存的に起こっている場合(転写共役因子の過剰発現，リン酸化による恒常的活性化，ER自身のリン酸化による活性化等々)，あるいはERは存在していてもそのER関連シグナルカスケードに非依存性になっている，すなわちその経路をスキップし，増殖進展に必要としなくなっている場合などである．

それぞれのケースで，抗エストロゲン剤は無効であるがアロマターゼ阻害剤は奏効が期待される場合，あるいはその反対の場合，両者ともに奏効しない場合と，内分泌療法の奏効性にもいろいろなパターンが予想され，その状況は患者ごとに異なり，極めて複雑であることが予想

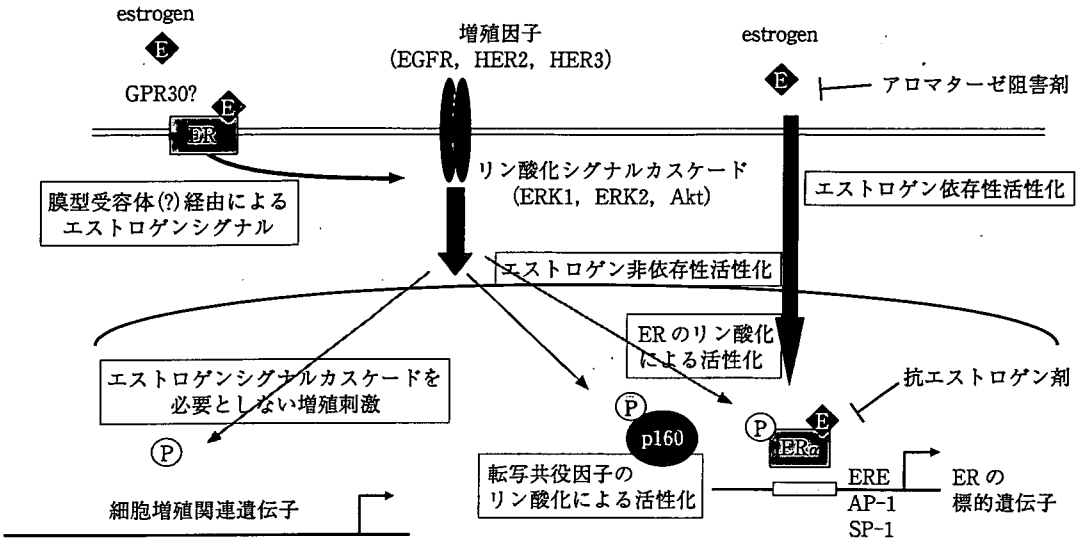


**図1 内分泌療法の感受性予測を困難にしている複数のER活性化機構**

される．抗エストロゲン剤の適応が良いのか，アロマターゼ阻害剤の適応が良いのか，あるいは具体的にどの薬剤が良いのか，患者ごとの正確な予測が可能な新たな予測因子が求められている．

## 4. 内分泌療法感受性予測の基礎研究

### a. 発現プロファイル解析による感受性予測

このような問題を解決する手段の一つとして，乳癌で発現が変動しているエストロゲン標的遺伝子を網羅的に捕らえて，そのプロファイルを解析することにより，患者ごとのエストロゲンシグナル経路がどのような状況にあるのか把握し，最適な治療法を見いだす，ということが考えられる．著者らは以前からこのような方法で内分泌療法の治療効果を詳細に予測することができないかという研究を行っている(図2)．本研究の詳細は原著[3,4]や他の総説[5,6]を参考にしていただきたい．現在，これまでに同定，抽出した候補遺伝子数十個を搭載した新しいタイプのマイクロアレイ(3次元アレイ)[7]を用いて臨床応用の可能性を試みているが，更に研究が進んで，数個の新規候補遺伝子によって予測が可能になれば，従来の免疫染色法，ないしはマルチプレックスRT-PCR法などのより簡便な手法によって奏効性予測が可能かもしれない．これまでの研究の過程で，HDAC6[8,9]やIGFBP4，EGR3[10]などのエストロゲン応答遺伝子で，臨床病理学的因子や予後などに関連する新規因子を同定してきた．今のところ，これらはそれぞれ単独でER/PgRを凌駕するような強力な予測因子ではないようだが，診断用コンテンツとしては有用であろうと考えている．更に，今後の研究の進展とともに新たな因子が見いだされる可能性も考えられる．

### b. アロマターゼ阻害剤の奏効性予測

閉経後の患者では卵巣機能の低下により血清中のエストロゲン濃度は低下するが，乳腺局所のエストロゲン濃度はある程度高いまま保たれている．現在閉経後内分泌療法の主流となりつつあるアロマターゼ阻害剤の作用機序は，局所でのアロマターゼを阻害して乳癌細胞へのエストロゲンの供給を遮断することである．癌細胞にもアロマターゼがあるという報告もあるが，大部分は腫瘍周辺の間質細胞に存在し，パラクリンにエストロゲンを供給している．ならば個々の乳癌に存在する間質の個性も治療効果に大きく影響するはずである．しかも癌細胞はエストロゲンだけでなく，他の増殖因子など，癌微小環境に存在する様々な因子によりその生存が