mass spectrometer (Applied Biosystems, Foster City, CA, USA) operated with electron spray ionization in the positive-ion mode, and the chromatographic separation was performed on Cadenza CD-C18 column (3×150 mm, 3.5 mm, Imtakt, Kyoto, Japan).

Laser-Capture Microdissection (LCM)/Microarray Analysis

Gene expression profiles of MBC and FBC cells were examined by microarray analysis. Four MBC and four FBC tissues were subjected to the study. LCM was conducted using the MMI Cellcut (Molecular Machines and Industries, Flughofstrase, Glattbrugg, Switzerland) according to previous reports [14, 16]. Briefly, breast carcinoma specimens (one specimen for each case) were embedded in Tissue-Tek optimal cutting temperature compound (Sakura Finetechnical Co., Tokyo, Japan), and serial sections were made at a thickness of 10 μm. Sections were stained with toluidine blue according to manufacturer's recommendation, and subsequently, breast carcinoma cells in each specimen (approximately 5,000 cells) were dissected under light microscopy and laser transferred from the serial sections. The total RNA (approximately 200 ng) was subsequently extracted from these cell fractions isolated by LCM using the RNeasy® Micro Kit (QIAGEN, Mannheim, Germany). Gene expression profiles were examined by microarray analyses. Whole Human Genome Oligo Microarray (G4112F, ID: 012391, Agilent Technologies), containing 41,000 unique probes, was used in this study, and sample preparation and processing were performed according to the manufacturer's protocol.

In our present study, we focused upon the expression profiles of two gene lists which were previously reported as estrogen-induced genes in FBC cell line MCF-7 [4, 5]. One was Frasor's list which consisted of 50 genes [4], and the other was Creighton's list which consisted of 63 genes [5]. If a gene was represented multiple times on the platform, the probe with strongest positive correlation with ESR1 (ERα) was selected. In order to compare the expression profiles of these genes, unsupervised hierarchical clustering analysis was performed using the Cluster and TreeView programs (the software copyright Stanford University 1998–1999, http://rana.stanford.edu) to generate tree structures based on the degree of similarity, as well as matrices comparing the levels of expression of individual genes in each specimens. Expression of genes was statistically evaluated by Student's t test, and $P<0.05$ was considered significant in this study.

Immunohistochemistry

The characteristics of primary antibody of aromatase [13], STS [17], and 17βHSD1 [15] were described previously. Monoclonal antibodies for ERα (ER1D5), ERβ (14C8), PR (MAB429), and Ki-67 (MIB1) were purchased from Immunotech (Marseille, France), Gene Tex (San Antonio, TX, USA), Chemicon (Temecula, CA, USA), and DAKO (Carpinteria, CA, USA), respectively. Rabbit polyclonal antibody for HER2 (A0485) was obtained from DAKO. Rabbit polyclonal antibody for receptor interacting protein 140 (RIP140) and retinoic acid receptor α (RARα) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

A Histofine Kit (Nichirei Biosciences, Tokyo, Japan), which employs the streptavidin-biotin amplification method, was used in this study. Immunoreactivity of estrogen-producing enzymes was detected in the cytoplasm, and the cases that had more than 10 % of positive cells were considered positive [18, 19]. Immunoreactivity of ERα, ERβ, PR, Ki-67, RIP140, and RARα was detected in the nucleus. These immunoreactivities were evaluated in more than 1,000 carcinoma cells, and subsequently, the percentage of immunoreactivity, i.e., labeling index (LI), was determined [20]. HER2 immunoreactivity was evaluated according to a grading system proposed in HercepTest (DAKO), and the cases with strongly circumscribed membrane staining of HER2 in more than 10 % carcinoma cells (i.e., score 3+) were considered positive in this study.

Results

Tissue Concentration of Estrogens and Androgens in MBC

We first examined tissue concentration of sex steroids in non-neoplastic male breast, MBC, and FBC tissues by LC-MS/MS. Median with minimum–max value of the estradiol level was 37.0 (8.0–74.0)pg/g in non-neoplastic male breast, 523 (267–633)pg/g in MBC, and 190 (15.7–540) pg/g in FBC (Fig. 1a). Tissue concentration of estradiol was significantly ($P=0.03$ and 14-fold) higher in MBC than non-neoplastic male breast tissues. Moreover, intratumoral estradiol concentration was 2.8-fold higher in MBC than in FBC tissues, although P value did not reach a significant level ($P=0.09$). On the other hand, tissue concentration of estrone was in 83.0 (56.0–359)pg/g in non-neoplastic male breast, 134 (67.0–280)pg/g in MBC, and 75.0 (13.0–555)pg/g in FBC, respectively, and the estrone level in MBC was not significantly different from that in non-neoplastic male breast or FBC ($P=0.72$ and $P=0.71$, respectively; Fig. 1b).

Tissue concentration of testosterone was high both in non-neoplastic male breast [1,519 (23.0–3,287)pg/g] and MBC [2,540 (1,454–3,483)pg/g], compared to that in FBC [133 (70.0–240)pg/g; $P=0.008$ in MBC vs. FBC], but no significant difference was detected between these two groups ($P=0.48$; Fig. 1c). Androstenedione has similar levels in these three groups [620 (53–7,525)pg/g in non-neoplastic male breast, 1,021 (291–1,805)pg/g in MBC, and 561 (160–5,785)pg/g in FBC] in this study (Fig. 1d).

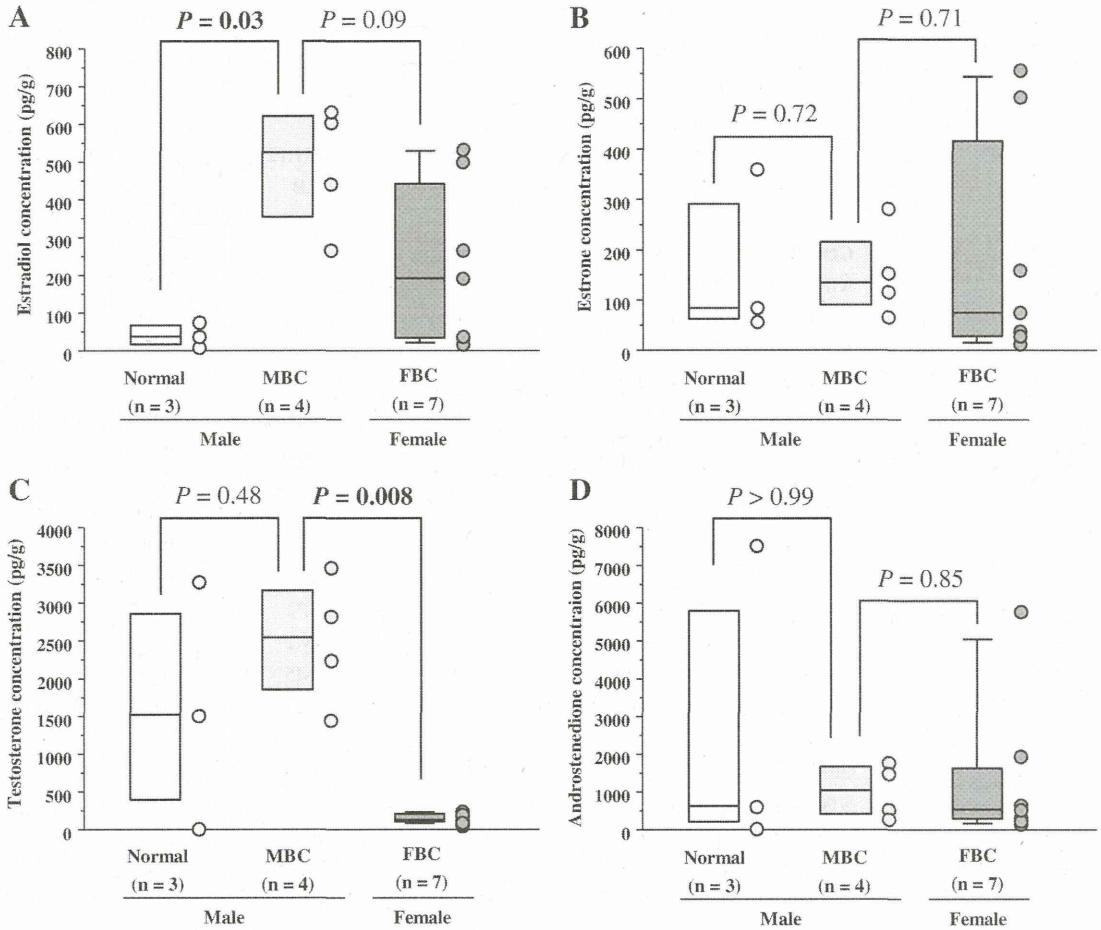

Fig. 1 Tissue concentration of estradiol (**a**), estrone (**b**), testosterone (**c**), and androstenedione (**d**) in non-neoplastic male breast, MBC, and FBC tissues. Each value was represented as a *circle*, and the grouped data were shown as *box-and-whisker plots*. The median value is demonstrated by a *horizontal line* in the box plot, and the *gray box* denotes the 75th (upper margin) and 25th percentiles of the values (lower margin). The *upper* and *lower bars* indicated the 90th and tenth percentiles, respectively. Statistical analysis was done by Mann–Whitney's *U* test; *P* values <0.05 were considered significant and indicated in *bold*

Expression Profiles of Estrogen-Induced Genes in MBC Compared with Those of FBC

We then performed microarray analysis in order to examine gene expression profiles of MBC cells isolated by LCM. Statistical analysis using Student's *t* test demonstrated that 12,295 probes showed significantly different expression between MBC and FBC cases. We then focused upon the expression profiles of two gene lists which were previously reported as estrogen-induced genes in FBC cell line MCF-7 (i.e., Frasor's list [4] and Creighton's list [5]) in order to examine molecular characteristics of estrogen actions in MBC. In the Frasor's list, 28 out of 50 (56 %) genes showed significantly different expression levels in MBC compared to FBC, and among these genes, 14 genes were highly expressed in MBC while 14 genes were lowly expressed (Table 1). In the Creighton's list, expression levels of 32 genes out of 63 (51 %) genes were significantly different between in MBC and FBC, and 18 genes were highly expressed in MBC while the other 14 genes were lowly expressed (Table 2). Five genes (RASGRP1, RARA, ADCY9, CXCL12, and NRIP1) were also included in these two gene lists, and expression levels of NRIP (P=0.0045) and ADCY9 (P=0.046) were significantly higher in MBC than FBC, and those of RARA (P=0.0012), RASGRP1 (P=0.011), and CXCL12 (P=0.012) were significantly lower in MBC.

As demonstrated in Fig. 2, results of unsupervised hierarchical cluster analysis revealed that MBC (n=4) and FBC cases (n=4) formed independent clusters regardless of the gene lists examined.

Immunolocalization of Estrogen-Producing Enzymes in MBC

We next immunolocalized estrogen-producing enzymes in 30 MBC tissues. Immunoreactivity of aromatase (Fig. 3a),

Table 1 List of genes identified as estrogen-induced genes by Frasor et al. (Frasor's list) [4]

Symbol	*P*	MBC vs. FBC	Symbol	*P*	MBC vs. FBC
CCND1	**0.041**	**L**	TGIF2	0.076	–
MYBL2	**0.027**	**L**	EGR3	0.36	–
RASGRP1[a]	**0.011**	**L**	**CXCL12**[a]	**0.012**	L
PKMYT1	0.13	–	GLRB	0.23	–
CBFA2T3	0.36	–	CHEK2	0.051	–
CDC20	**0.046**	**L**	FOS	0.056	–
IGFBP5	0.18	–	SLK	0.056	–
CCBP2	**0.0064**	**L**	**ELL2**	**<0.0001**	**H**
MYC	**0.015**	**L**	**RFC4**	**0.0084**	**H**
CCNA2	**0.0097**	**L**	**ADCY9**[a]	**0.046**	**H**
POLE2	**0.019**	**L**	**MYB**	**0.011**	**H**
BRCA2	**0.022**	**L**	**BIRC5**	**0.047**	**H**
RARA[a]	**0.0012**	**L**	**NRIP1**[a]	**0.0045**	**H**
HOXC5	**0.0043**	**L**	**MCM3**	**0.0021**	**H**
CALCR	**0.0023**	**L**	**RBBP7**	**0.0031**	**H**
POLA2	**0.011**	**L**	**RAB31**	**0.0022**	**H**
AREG	**0.0021**	**H**	WISP2	0.52	–
PCNA	**0.0093**	**H**	MCM2	0.52	–
OSTF1	**0.0039**	**H**	MCM5	0.31	–
GADD45B	**0.048**	**H**	CDC2	0.051	–
VEGF	0.27	–	AURKA	0.33	–
PPP2R1B	0.30	–	BUB1	0.76	–
STC2	**0.020**	**H**	TMF1	0.66	–
TSPAN5	0.088	–	CDC6	0.81	–
IGFBP4	0.12	–	JAK1	0.96	–

Comparison of gene expression between MBC and FBC was performed by Student's *t* test. $P < 0.05$ was considered positive and described as *boldface*

"H" means that the gene is highly expressed in MBC compared to FBC, and "L" means that the gene is lowly expressed in MBC compared to FBC

[a]Genes contained by both Frasor's and Creighton's lists

STS (Fig. 3b), and 17βHSD1 (Fig. 3c) was detected in the cytoplasm of carcinoma cells in MBC tissues, but STS immunoreactivity was weaker and focal. The number of positive cases was as follows: aromatase, 19/30 (63 %); STS, 2/30 (6.7 %); and 17βHSD1, 20/30 (67 %). Non-neoplastic mammary glands and intratumoral stroma were negative for aromatase (Fig. 3d), STS, and 17βHSD1 in this study.

Immunolocalization of ERs and Estrogen-Induced Genes in MBC Compared with FBC

We also evaluated an association of several immunohistochemical parameters between MBC ($n=30$) and FBC tissues ($n=72$). As shown in Table 3, ERα and ERβ LIs were significantly ($P<0.0001$ and $P=0.001$) higher in MBC than FBC. When cases with ER LI of 10 % were considered ER-positive breast carcinoma [17, 18], all MBC cases examined were positive for ERα, while 67 % (48/72) of FBC were positive for ERα. In addition, a great majority (77 %) of MBC cases showed double positive for ERα and ERβ, and its frequency was significantly ($P=0.0009$) higher than that in FBC (39 %). PR LI was also significantly ($P=0.011$) higher in MBC than FBC, and it was positively associated with ERα LI [$P=0.03$ and $r^2=0.16$ (data not shown)]. On the contrary, Ki67 LI was significantly ($P=0.019$) lower in MBC than FBC. HER2 status was not significantly different between these in this study.

Since our microarray analyses demonstrated different expression profiles of estrogen-induced genes in MBC from those in FBC (Fig. 2), we also performed immunohistochemistry for two representative genes included in both Frasor's and Creighton's lists [RARA (RARα) and NRIP1 (RIP140)] to confirm the results. RARα immunoreactivity was sporadically detected in the nuclei of MBC cells (Fig. 4a), and its LI was significantly ($P=0.0034$ and 0.62-fold) lower in MBC than FBC (Fig. 4b). On the other hand, RIP140 immunoreactivity was frequently detected in the nuclei of MBC cells (Fig. 4c), and RIP140 LI in MBC was significantly ($P=0.002$ and 1.91-fold) higher than FBC (Fig. 4d).

Discussion

To the best of our knowledge, this is the first study to have demonstrated intratumoral estrogen concentrations in MBC tissues. In the present study, tissue concentration of estradiol

Table 2 List of genes identified as estrogen-induced genes by Creighton et al. (Creighton's list) [5]

Symbol	*P*	MBC vs. FBC	Symbol	*P*	MBC vs. FBC
ATAD2	**0.0074**	**L**	PAK1IP1	0.61	–
CISH	0.056	–	CA12	0.80	–
GREB1	0.051	–	MYBL1	0.23	–
RASGRP1[a]	**0.011**	**L**	IRS1	0.37	–
ADSL	**0.0048**	**L**	KLF10	0.94	–
FLJ22624	**0.026**	**L**	**ADCY9**[a]	**0.046**	**H**
IGF1R	**0.015**	**L**	**FLJ11184**	**0.0064**	**H**
BRIP1	**0.0079**	**L**	**TIPARP**	**0.0045**	**H**
IL17RB	**0.0082**	**L**	TPBG	0.076	–
TEX14	**0.0004**	**L**	ZWILCH	0.25	–
PLK4	**0.012**	**L**	**MCM4**	**0.046**	**L**
RARA[a]	**0.0012**	**L**	**CXCL12**[a]	**0.012**	**L**
PTGES	0.066	–	**DSU**	**0.024**	**L**
SNX24	**0.016**	**L**	OLFM1	0.11	–
HSPB8	0.38	–	EEF1E1	0.43	–
TFF1	0.45	–	LOC56902	0.079	–
SIAH2	0.25	–	**NOL7**	**0.041**	**H**
OGFOD1	0.83	–	**SDCCAG3**	**0.030**	**H**
WDHD1	0.32	–	**PPIF**	**0.0046**	**H**
ZNF259	0.50	–	**MRPS2**	**0.024**	**H**
SLC39A8	0.83	–	**ALG8**	**0.0066**	**H**
WHSC1	0.63	–	**SLC9A3R1**	**0.014**	**H**
CTNNAL1	0.17	–	**XBP1**	**0.021**	**H**
DLEU1	0.18	–	CSPP1	0.76	–
FER1L3	**0.019**	**H**	THBS1	0.66	–
LRRC54	**0.024**	**H**	ENST00000379534	0.90	–
SGK3	**0.0068**	**H**	ENST00000278505	0.35	–
CTPS	**0.0059**	**H**	PPAT	0.61	–
LRP8	0.054	–	**MYB**	**0.029**	**H**
FHL2	**0.0005**	**H**	THRAP2	0.20	–
NRIP1[a]	**0.0045**	**H**	TPD52L1	0.57	–
DNAJC10	**0.042**	**H**			

Comparison of gene expression between MBC and FBC was performed by Student's *t* test. $P<0.05$ was considered positive and described as *boldface*

"H" means that the gene is highly expressed in MBC compared to FBC, and "L" means that the gene is lowly expressed in MBC compared to FBC

[a]Genes contained by both Frasor's and Creighton's lists

was significantly higher (14-fold) in MBC [523 (267–633) pg/g] than the non-neoplastic male breast tissues (Fig. 1a), whereas estrone, testosterone, and androstenedione levels did not significantly change between in these two groups (1.6-fold, 0.83-fold, and 1.6-fold, respectively). Serum estradiol concentration in men is known to be similar to that in postmenopausal women [21]. Chetrite et al. [22] previously showed that estradiol level was significantly higher in breast carcinomas in postmenopausal women [388±106 pg/g (mean±SEM)] than in the areas considered as morphologically normal in the same patients, which is currently explained by intratumoral production of estradiol [3]. Although serum estradiol level in MBC patients has been reported twofold higher than that in healthy subjects [23], our present results suggest possible local production of estradiol in MBC tissues as well as FBC.

In the breast carcinoma of postmenopausal women, intratumoral estradiol is produced by aromatase and/or STS pathways [24]. In our present study, aromatase immunoreactivity was detected in 63 % of MBC cases. Its frequency was in good consistent with a previous report [13], and similar to that in FBC reported previously (55–77 %) [25, 26]. The positivity of 17βHSD1 immunoreactivity in MBC in our present study (67 %) was also similar to previous reports in FBC (47–61 %) [27, 28]. On the other hand, STS immunoreactivity was detected only in 7 % of MBC cases in this study, which was much lower (approximately 0.1-fold) than that in FBC reported (60–90 %) [29, 30]. Therefore, it is suggested that estradiol is mainly synthesized by aromatase pathway in MBC rather than STS.

Results of our present study also showed that estradiol concentration was 2.8-fold higher in MBC than postmenopausal

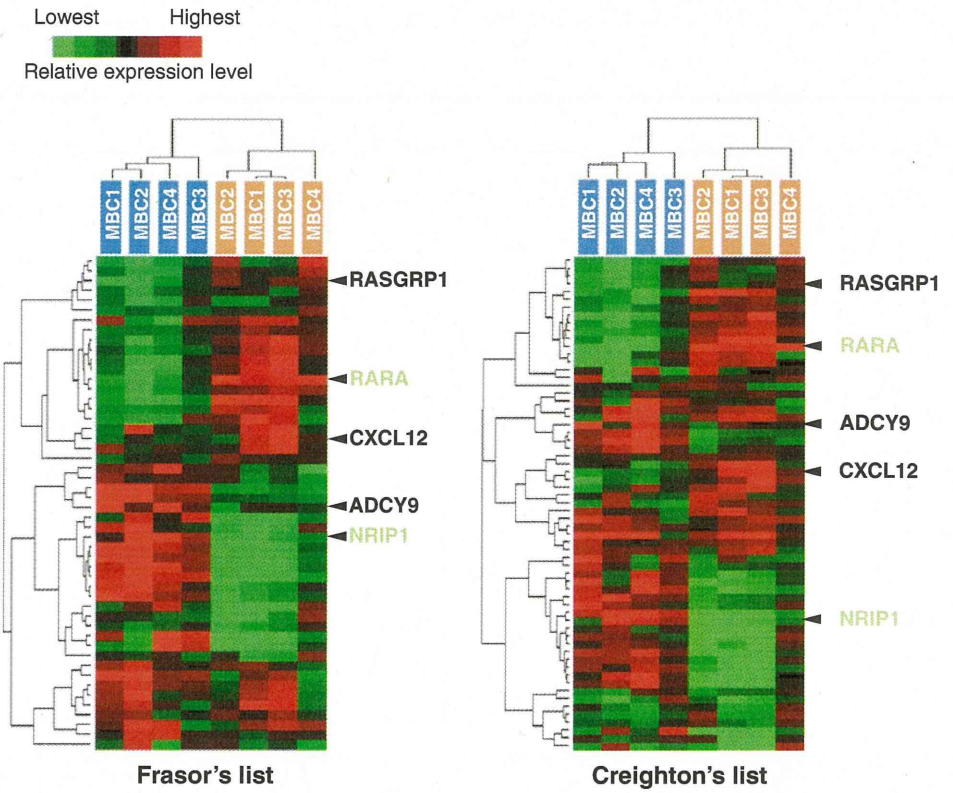

Fig. 2 Unsupervised hierarchical clustering analysis of mRNA expression levels focused on the genes which were previously reported as estrogen-induced genes [Frasor's list (*left*; 50 genes) and Creighton's list (*right*; 63 genes)]. Eight breast carcinoma samples [four MBCs (MBC1-4) and four FBCs (FBC1-4)] were used in this study, and genes and/or cases were grouped according to the similarity of gene expression, and the *shorter length of the branch* represents the higher similarity of cluster pairs. *Color of blocks* represents relative mRNA expression level of each gene, compared to the average in eight breast carcinoma samples. Five genes included in both lists (i.e., RASGRP1, RARA, ADCY9, CXCL12, and NRIP1) were indicated by *wedge*. Among these, two genes (RARA and NRIP1), which were subsequently evaluated by immunohistochemistry, were *highlighted in green*

FBC. Previously, Sonne-Hansen and Lykkesfeldt [31] reported that aromatase preferred testosterone as a substrate in MCF-7 breast carcinoma cells. In addition, plasma concentration of testosterone is approximately tenfold higher in men than postmenopausal women, while that of androstenedione is approximately1.5-fold

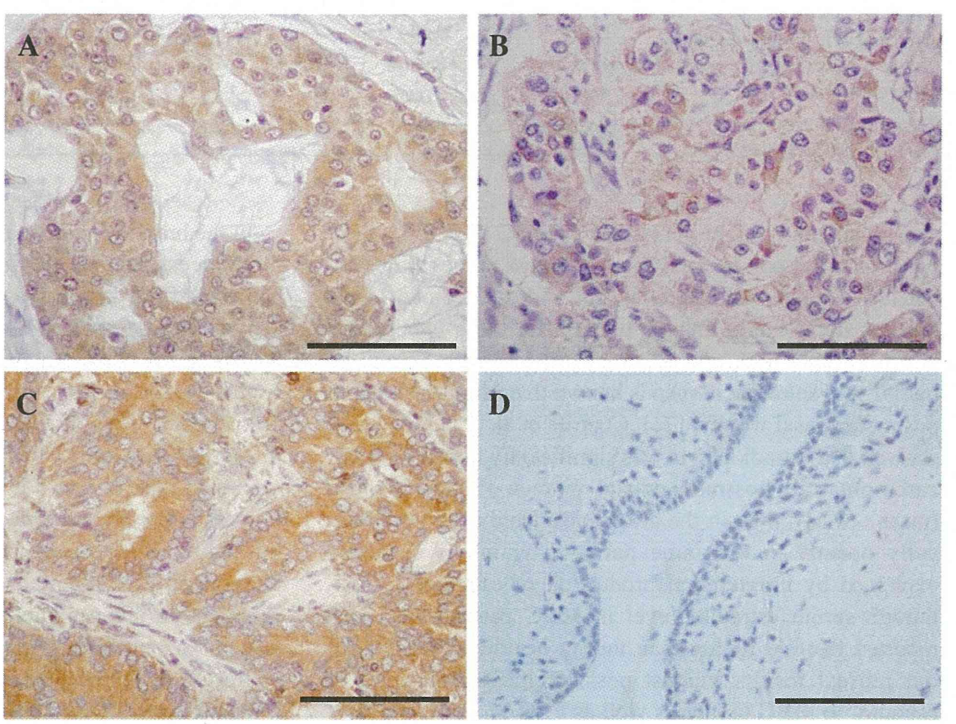

Fig. 3 Immunohistochemistry of estrogen-producing enzymes in MBC tissues. Immunoreactivity for aromatase (**a**), STS (**b**), and 17βHSD1 was visualized with 3,3′-diaminobenzidine (DAB; *brown*) and detected in the cytoplasm of carcinoma cells. Aromatase immunoreactivity was not detected in non-neoplastic mammary gland or stroma (**d**). *Bar*=100 μm, respectively

Table 3 Immunohistochemical features of MBC compared with FBC

	MBC n=30	FBC n=72	P value
ERα LI (%)[a]	90.5 (43–98.0)	40.0 (0.0–92)	**<0.0001**
ERα status			
Positive	30 (100 %)	48 (67 %)	
Negative	0 (0 %)	24 (33 %)	**<0.0001**
ERβ LI (%)[a]	27.5 (0–95)	8.5 (0–72)	**0.001**
ERβ status			
Positive	23 (77 %)	35 (49 %)	
Negative	7 (23 %)	37 (51 %)	**0.017**
ERα/ERβ status			
Positive/positive	23 (77 %)	28 (39 %)	
Others	7 (23 %)	44 (61 %)	**0.0009**
PR LI (%)[a]	43.5 (6–95)	17.5 (0–93)	**0.011**
HER2			
Positive	5 (17 %)	24 (33 %)	
Negative	25 (83 %)	48 (67 %)	0.099
Ki67 LI (%)[a]	15.5 (1.0–30)	20.0 (2.0–67)	**0.019**

[a] Data was presented as median with minimum–max or the number of cases with percentage. P value <0.05 was considered significant and described as *boldface*

higher in men [21]. Therefore, estradiol may be mainly produced from circulating testosterone by aromatase in MBC tissues. These findings also suggest that aromatase inhibitors are possibly effective in a selective group of MBC patients. A phase 2 trial used aromatase inhibitor, and GnRH analogue (SWOG-S 0511 trial) is currently ongoing in MBC patients [32].

The biological effects of estrogens are mediated through an initial interaction with ERα and/or ERβ, and ERs functions as hetero- or homodimers. In this study, both ERα and ERβ were more frequently immunolocalized in MBC than in FBC, which was in good agreement with previous reports [10–12]. Moreover, we also found that a great majority (77 %) of MBC cases showed double positive for ERα and ERβ, and its frequency was significantly (2.0-fold) higher than FBC cases (Table 1). Therefore, it may be possible to speculate that ERs are frequently heterodimerized in MBC tissues. Heterodimerization of ERα and ERβ modulates biological functions of each ER [33, 34], and FBC patients double positive for ERα and ERβ had longer disease-free and overall survival than those showed positive for ERα only [35, 36]. On the other hand, Weber-Chappuis et al. [37] suggested that functions of ER in MBC were different from that in FBC, and Johansson et al. [38] recently demonstrated that MBC was classified into two groups (i.e., luminal M1 and M2), those differed from the intrinsic subtypes of ER-positive FBC, by microarray analyses. Therefore, estrogen actions in MBC may not be necessarily the same as those in FBC, which is partly due to the different ERα/ERβ status from FBC.

Results of our microarray analysis did demonstrate that a majority of estrogen-induced genes (56 % in Frasor's list and 51 % in Creighton's list) showed significantly different expression between in MBC and FBC, and MBC cases formed a different cluster from FBC cases. We also confirmed these results by employing immunohistochemistry for representative genes (i.e., RARα and RIP140). Therefore, it is reasonably postulated that molecular functions of estrogens in MBC may be different from those in FBC based on the results above. However, it is also true that estrogen-induced genes examined in this study were identified in female breast cancer cell line MCF-7, and it is still not clarified whether these genes were similarly regulated by estrogen in MBC tissues or not, which also suggests that all the genes detected at markedly different levels in MBC compared to FBC were therefore not necessarily regulated by estrogens. In addition, only two genes on Creighton's list (CA12 and SIAH2) were included in the gene list, which was recently identified as MBC-specific genes by Johansson et al. [38]. Estrogen-induced genes are not determined yet in MBC because of unavailability of appropriate cell line and/or its relevant in vivo model. Therefore, further examinations are required to clarify the molecular features of estrogen actions in MBC.

Among the genes overexpressed in FBC (summarized in Tables 1 and 2), MYC (C-MYC) was well known to be associated with poor prognosis or adverse clinical outcome of ER-positive breast cancer patients [39], and RARA (RARα) upregulated 17βHSD1 and contributed to in situ production of estradiol in FBC [40]. IGF1R (insulin-like growth factor receptor) has been considered to promote breast carcinoma cell growth by interacting with estrogen signaling [41]. In addition, Ma et al. and Wang et al. independently reported that IL17RB (interleukin-17 receptor B) expression was significantly associated with increased risks of recurrence in ERα-positive breast cancer patients [42, 43]. However, among the genes highly expressed in MBC, MYB (c-myb) was associated with a good prognosis in the patients [44]. NRIP1 (RIP140) is a negative transcriptional regulator of hormone receptor [45, 46] and inhibited ERα activity in the breast carcinoma cells [43]. RBBP7 (RBAP46) also modulated estrogen responsiveness in breast carcinoma cells through an interaction with ERα [47] and inhibited an estrogen-stimulated progression of transformed breast epithelial

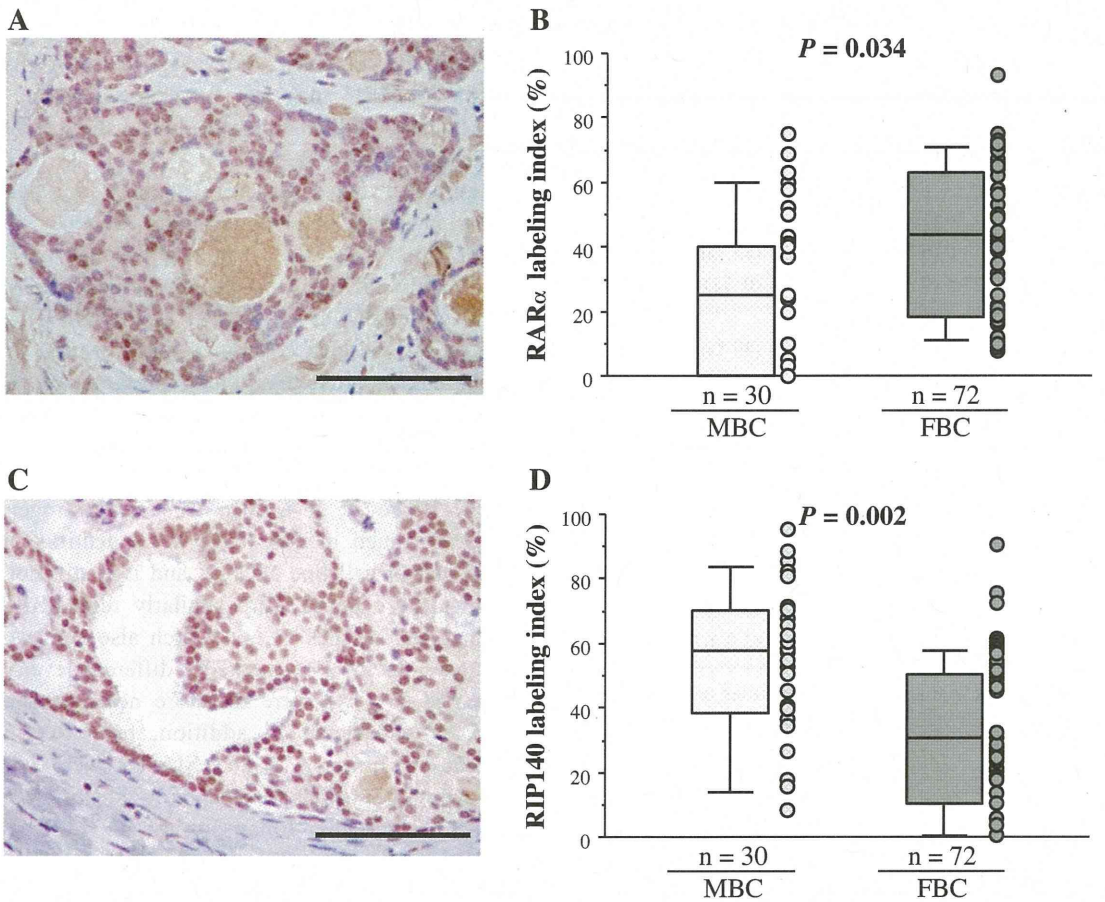

Fig. 4 Immunohistochemistry of RARα (**a**, **b**) and RIP140 (**c**, **d**) in MBC tissues. RARα (**a**) and RIP140 (**c**) immunoreactivity was visualized with DAB (*brown*) and detected in the nuclei of carcinoma cells. *Bar*=100 μm, respectively. Relative immunoreactivity of RARα and RIP140 was summarized in **b** and **d**, respectively. Each value was represented as a *circle*, and the grouped data were shown as *box-and-whisker plots*. The median value is demonstrated by a *horizontal line* in the box plot, and the *gray box* denotes the 75th (upper margin) and 25th percentiles of the values (lower margin). The *upper* and *lower bars* indicate the 90th and tenth percentiles, respectively. Statistical analysis was performed by Mann–Whitney's *U* test; *P* values <0.05 were considered significant and indicated in *bold*

cells [48]. In addition, FHL2 (four and a half LIM domains 2) was reported to inhibit proliferation and invasion of breast carcinoma cells by suppressing the function of ID3 (inhibitor of DNA binding 3), which was also known as one of the adverse prognostic factor of patients with breast cancer [49, 50]. Considering the functions of these gene above, estrogens may more efficiently promote aggressive clinical behavior in FBC than MBC, although some genes highly expressed in MBC were indeed associated with aggressive phenotypes of the breast carcinoma, such as AREG (amphiregulin) and XBP1 (X-box binding protein 1) [51, 52]. To date, tamoxifen is used as an endocrine therapy for MBC patients. However, it has been reported that expression profile of estrogen responsive gene was closely related to the response to tamoxifen in FBC patients [53]. Further examinations are required to clarify molecular functions of estrogen actions in MBC to improve the effectiveness of endocrine therapy for MBC patients.

In summary, intratumoral concentration of estradiol was significantly higher in MBC than non-neoplastic male breast tissues in this study, and aromatase and 17βHSD1 were frequently immunolocalized in MBC tissues. In addition, a great majority (77 %) of MBC cases showed positive for both ERα and ERβ, and its frequency was significantly higher than FBC cases. Results of microarray analysis revealed that expression profiles of genes known to be regulated by estrogen were markedly different between MBC and FBC. These results suggest that estradiol is mainly produced by aromatase from circulating testosterone in MBC tissues, and expression profiles of estrogen-induced genes in MBC are different from FBC, which may be partly due to their different ERα/ERβ status.

Acknowledgments We appreciate the skillful technical assistance of Mr. Katsuhiko Ono (Department of Anatomic Pathology, Tohoku University Graduate School of Medicine).

Disclosures The authors declare that there is no conflict of interest to be disclosed.

Funding This work was partly supported by Grant-in-Aid for Scientific Research (24790343) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

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Breast Cancer Res Treat (2013) 139:731–740
DOI 10.1007/s10549-013-2595-x

PRECLINICAL STUDY

Androgen metabolite-dependent growth of hormone receptor-positive breast cancer as a possible aromatase inhibitor-resistance mechanism

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Received: 10 May 2013 / Accepted: 1 June 2013 / Published online: 19 June 2013
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Abstract Aromatase inhibitors (AIs) have been reported to exert their antiproliferative effects in postmenopausal women with hormone receptor-positive breast cancer not only by reducing estrogen production but also by unmasking the inhibitory effects of androgens such as testosterone (TS) and dihydrotestosterone (DHT). However, the role of androgens in AI-resistance mechanisms is not sufficiently understood. 5α-Androstane-3β,17β-diol (3β-diol) generated from DHT by 3β-hydroxysteroid dehydrogenase type 1 (HSD3B1) shows androgenic and substantial estrogenic activities, representing a potential mechanism of AI resistance. Estrogen response element (ERE)-green fluorescent protein (GFP)-transfected MCF-7 breast cancer cells (E10 cells) were cultured for 3 months under steroid-depleted, TS-supplemented conditions. Among the surviving cells, two stable variants showing androgen metabolite-dependent ER activity were selected by monitoring GFP expression. We investigated the process of adaptation to androgen-abundant conditions and the role of androgens in AI-resistance mechanisms in these variant cell lines. The variant cell lines showed increased growth and induction of estrogen-responsive genes rather than androgen-responsive genes after stimulation with androgens or 3β-diol. Further analysis suggested that increased expression of HSD3B1 and reduced expression of androgen receptor (AR) promoted adaptation to androgen-abundant conditions, as indicated by the increased conversion of DHT into 3β-diol by HSD3B1 and AR signal reduction. Furthermore, in parental E10 cells, ectopic expression of HSD3B1 or inhibition of AR resulted in adaptation to androgen-abundant conditions. Coculture with stromal cells to mimic local estrogen production from androgens reduced cell sensitivity to AIs compared with parental E10 cells. These results suggest that increased expression of HSD3B1 and reduced expression of AR might reduce the sensitivity to AIs as demonstrated by enhanced androgen metabolite-induced ER activation and growth mechanisms. Androgen metabolite-dependent growth of breast cancer cells may therefore play a role in AI-resistance.

Electronic supplementary material The online version of this article (doi:10.1007/s10549-013-2595-x) contains supplementary material, which is available to authorized users.

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