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H. 知的財産権の出願・登録状況

1. 特許取得
   無し。
2. 実用新案登録
   無し。
3. その他
   無し。

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

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研究成果の刊行物・別刷

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Original Article

Rim Enhancement of Breast Cancers on Contrast-Enhanced MR Imaging: Relationship with Prognostic Factors

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Background: There is little evidence regarding associations between magnetic resonance imaging (MRI) features and other important histopathological prognostic factors of breast cancer. The purpose of our study was to investigate the relationship between rim enhancement on MRI and common histopathological prognostic factors of breast cancers.

Methods: We reviewed the contrast-enhanced MR images of 106 consecutive women with histopathologically verified invasive breast carcinomas. Three radiologists assessed the images of each lesion for the presence of rim enhancement on early and delayed images, which were classified into four patterns. Statistical analyses were performed to explore the associations of these patterns with common histopathological prognostic factors and patient age.

Results: Positive ratios of lymph node metastasis and blood vessel invasion and negative ratios of hormone receptors were higher in the invasive cancers with rim enhancement than those without rim enhancement. Rim enhancement was more frequent in invasive ductal cancers with a higher histological grade and larger invasive cancers. The pattern of rim enhancement with centripetal progression showed a significantly increased risk of lymph node metastasis and was associated with a larger size of invasive lesion when compared with the other patterns. Invasive cancers with rim enhancement and little change between the early and delayed images and with centrifugal progression showed significantly less hormone receptor positivity than those without rim enhancement.

Conclusions: Rim enhancement patterns of breast cancers on contrast-enhanced MRI are related to common histopathological prognostic factors and these patterns may be valuable in the preoperative evaluation of breast cancers.

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Key words: Breast cancer, MRI, Rim enhancement, Prognostic factor

Recently, contrast-enhanced magnetic resonance imaging (MRI) has been playing an important part in the detection and estimation of the extent of breast cancer[1-4]. However, the role of contrast-enhanced MRI is still unclear in the evaluation of the biological activity of breast cancer. Rim enhancement is known as a fairly specific sign of breast cancer on contrast-enhanced MRI of breast masses[2,5-7], but it is not always seen in cases of breast cancer. Our goal was to explore the associations between the rim enhancement of invasive breast cancer on dynamic contrast-enhanced MRI and common histopathological prognostic factors used in breast cancer, and to examine whether the rim enhancement patterns are useful in predicting tumor biological activity preoperatively.

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Abbreviations:
MRI, Magnetic resonance imaging; MR, Magnetic resonance; ER, Estrogen receptor; PgR, Progesterone receptor; OR, Odds ratio; CI, Confidence interval; ANOVA, One-way analysis of variance; NPV, Negative predictive value; PPV, Positive predictive value; ROI, Region of interest; PCNA, Proliferating cellular nuclear antigen; VEGF, Vascular endothelial growth factor

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Materials and Methods

Patients

We reviewed the magnetic resonance (MR) images of 106 consecutive female patients (age, 27-78 years, mean age 56 years) with histopathologically verified invasive breast carcinomas. After informed consent, they underwent contrast-enhanced dynamic MR mammography before open biopsy and neoadjuvant chemotherapy from April to September 2001. Initially the lesions were detected by physical examination, mammography, or ultrasonography. The histopathologic diagnoses were invasive ductal carcinoma (n = 95) (35 papillotubular carcinomas, 12 solid-tubular carcinomas, 48 scirrhous carcinomas), and others (n = 11) including mucinous carcinoma (n = 6), invasive lobular carcinoma (n = 2), adenoid cystic carcinoma (n = 1), apocrine carcinoma (n = 1), and tubular carcinoma (n = 1).

MR Imaging

MR imaging (MRI) was performed at 1.5 T (Signa; GE Medical Systems, Milwaukee, WI, U.S.A). The tumor-bearing breast in each patient was examined with a dedicated breast coil with the patient in the prone position.

Before administration of contrast material, transverse T1-weighted [500/10 (repetition time msec/echo time msec)] spin-echo imaging and sagittal T2-wighted (4000/84.1) fast spin-echo imaging with fat saturation were performed. The images were obtained using a 32-cm field of view with 9-10-mm thick sections, 2 mm gaps, and a 512 × 224 matrix with two or three signals acquired.

Then dynamic contrast-enhanced images were obtained using a 3D time of flight-first spoiled gradient-recalled echo sequences (7.5/1.7; inversion time 30 msec, flip angle, 15°), a 16-18-cm field of view, 512 × 224 matrix, and 4-6-mm section thickness and no interval gaps. The sagittal or coronal whole breast images were obtained three times, once before and twice after intravenous bolus injection of gadopentetrate dimeglumine (Magnevist; Nihon Schering, Osaka, Japan) at a dose of 0.1 mmol per kilogram of body weight within 10-15 seconds, followed by a 20-mL saline solution flush. The time interval for sequencing was 100 seconds and 300 seconds after administration of contrast agent and each acquisition time was approximately 90 seconds.

Late transverse T1-weighted (850/10) spin-echo imaging with fat saturation was performed after the dynamic study.

Image Analysis

Postprocessing subtraction and black-and-white reversion of dynamic images were performed in all patients. We obtained two different series of subtracted images for each patient. Images obtained before the administration of contrast material were subtracted from early phase (100 seconds) images and delayed phase (300 seconds) images obtained after administration of contrast material, respectively. All subtracted images were assessed by three radiologists (M.J., Y.K., K.K.) together by means of consensus. Contrast medium enhancement on both early and delayed images were classified into "rim" and "entire" patterns. The rim pattern meant that the periphery was more enhanced than the center in a tumor lesion. The "entire" pattern was homogenous enhancement of the tumor. From the changes in the patterns between early and delayed images, four changes in enhancement were observed as follows (Fig 1):

A. Entire enhancement in both early and delayed images. This pattern had no rim enhancement.

B. Rim enhancement in the early image, followed by little change in the delayed image.

C. Rim enhancement in the early image, followed by progressive central filling of contrast medium in the delayed image (centripetal progression of enhancement).

D. Rim enhancement which was clearer in the delayed image than in the early image as a result of central washout of contrast medium (centrifugal progression of enhancement).

In cases of multifocal or multicentric disease showing both entire and rim enhancement patterns, rim enhancement patterns were classified. We visually assessed rim and entire enhancements and changes in the enhancements between early and delayed images. The transverse T1-weighted spin-echo images, sagittal T2-wighted fast spin-echo images and postcontrast T1-weighted spin-echo images after the dynamic study were not used for the study.

Histopathologic Analysis

The histologic materials were stained with

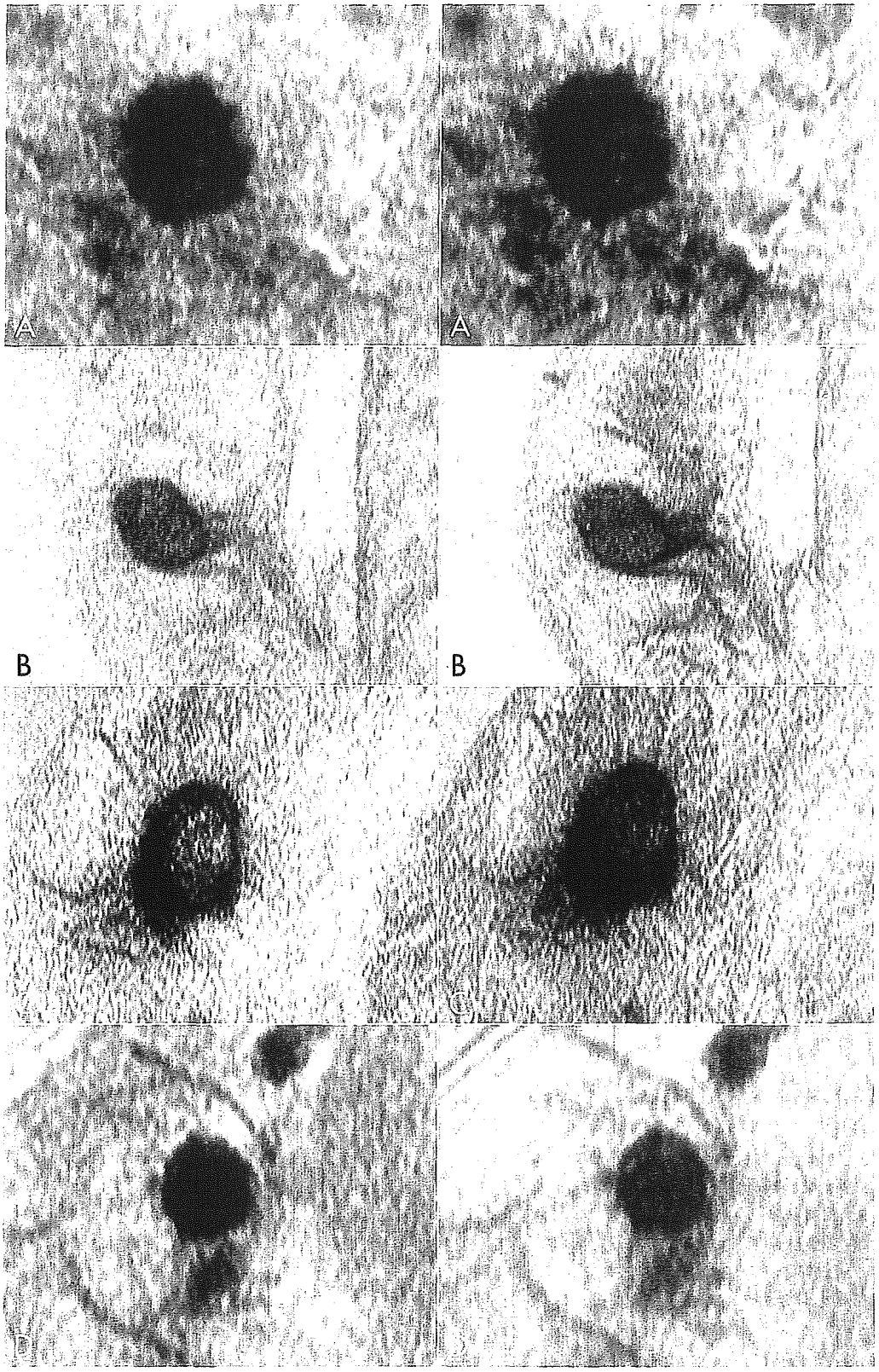

Fig 1. Four patterns changing from early (left) to delayed (right) subtracted and black-and-white reversed 3D-TOF-FSPGR images. (A) Pattern A. Entire enhancement in both early and delayed images. (B) Pattern B. Rim enhancement in the early image, followed by little change in delayed image. (C) Pattern C. Rim enhancement in the early image, followed by progressive central filling of contrast medium in the delayed image. (D) Pattern D. Rim enhancement which was clearer in the delayed image than in the early image as a result of central washout of contrast medium.

hematoxylin and eosin. The histologic interpretations were made by pathologists, without knowledge of the MR imaging findings. All breast cancers were histologically classified according to the criteria of the Japanese General Rules for Clinical and Pathological Recording of Breast Cancer[8]. The histological grade was determined using the modified Bloom and Richardson' criteria[9]. The size of the tumor was microscopically determined as the largest dimension of the invasive lesion in mm. Steroid hormone receptor status was determined by the immunohistochemical analysis. Blood vessel invasion was defined as penetration by the tumor into the lumen of an artery or vein and the presence of blood vessel invasion was recorded. The number of axillary lymph node metastases was recorded.

Immunohistochemical Examination

Tumor specimens were fixed in 10% neutrally buffered formalin and embedded in paraffin. The avidin-biotin-peroxidase method was used for immunohistochemical analysis. Sections 3 μm thick were dewaxed and dehydrated on silanized glass slides. A microwave oven was used for antigen retrieval. Endogenous peroxidase activity was blocked by incubation in hydrogen peroxide. Nonspecific binding was blocked with 10% nonimmune serum. The slides were then incubated in phosphatate-buffered saline with specific monoclonal antibodies, anti-estrogen receptor (1D5, DAKO, Japan) and anti-progesterone receptor (PgR636, DAKO) for 30 min, at room temperature. Normal mouse serum was substituted for primary antibodies as a negative control. Cases were considered estrogen receptor (ER) or progesterone receptor (PgR) positive if nuclear staining appeared in 10% or more of tumor cells.

Statistical Methods

Chi-square test or logistic regression analysis was used to explore the association between rim enhancement and the following prognostic factors: (1) histopathologic type (papillotubular carcinoma, solid-tubular carcinoma, scirrhous carcinoma and others), (2) lymph node metastasis (absence or presence), (3) blood vessel invasion (absence or presence), (4) histological grade (Grades 1-3), (5) invasive size of tumor ($\leqq$20 mm, 21 mm-50 mm and $\geqq$51 mm), (6) ER or PR status (either or both positive and both negative) and (7) patient age ($\leqq$50 and $>$50). Further statistical analyses were performed to explore the associations between the patterns of rim enhancement (A, B, C and D) and prognostic factors in detail. As for hormone receptors, lymph node metastasis and tumor size, odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) were obtained by logistic regression models using each prognostic factor as a dependent variable. One-way analysis of variance (ANOVA) was used to explore the association of the rim enhancement pattern with tumor size and patient age. Fisher's exact test was used to explore the association between the rim enhancement pattern and blood vessel invasion. A p-value less than 0.05 was considered statistically significant. A statistical program, STATA version 8.1 (STATA Corp., TX, USA), was used.

Results

The association between the presence of rim enhancement and each prognostic factor is summarized in Table 1. Rim enhancement was seen in 55 (52%) of 106 patients with breast cancer. Five factors (lymph node metastasis, blood vessel invasion, histological grade, tumor size and hormone receptor) correlated with rim enhancement, but there was no correlation between rim enhancement and histopathologic type or patient age.

1) The axillary lymph nodes were sampled, and 42 of 105 (40%) patients were found to be positive for metastasis. The positivity rate of lymph node metastasis was significantly higher in the group with rim enhancement ($p=0.017$). Rim enhancement had a 72% (36/50) negative predictive value (NPV) and a 51% (28/55) positive predictive value (PPV) for lymph node metastasis.

2) Rim enhancement had a 100% (51/51) NPV for blood vessel invasion. Although the PPV was 15% (8/55), the association between rim enhancement and blood vessel invasion was statistically significant ($p=0.005$).

3) Histological grading was possible for 95 invasive ductal carcinomas. As the histological grade increased, the rate of rim enhancement became higher ($p<0.001$).

4) As the tumor became larger in size, the rate of rim enhancement became higher ($p<0.001$).

5) The absence of rim enhancement had a 96% (49/51) PPV for the presence of hormone receptors. Although the NPV was 29% (16/55), the association between rim enhancement and hormone receptor was statistically significant ($p<0.001$).

Table 1. Associations Between Rim Enhancement and Prognostic Factors in Breast Cancers

Prognostic factors	Rim enhancement		P-value
	− (%)	+ (%)	
Histopathologic diagnosis			
Papillotubular carcinoma	18 (35)	17 (31)	0.13
Solid-tubular carcinoma	2 (4)	10 (18)	
Scirrhous carcinoma	26 (51)	22 (40)	
Others	5 (10)	6 (11)	
Lymph node metastasis			
Negative	36 (72)	27 (49)	0.017
Positive	14 (28)	28 (51)	
Blood vessel invasion			
Negative	51 (100)	47 (85)	0.005
Positive	0 (0)	8 (15)	
Histological grade			
Grade 1	7 (14)	0 (0)	P for trend < 0.001*
Grade 2	35 (69)	31 (56)	
Grade 3	4 (9)	18 (44)	
Tumor size (mm)			
-20	30 (58)	11 (20)	P for trend < 0.001*
21-50	19 (37)	39 (71)	
51-	2 (4)	5 (9)	
Hormone receptor			
Negative	2 (4)	16 (29)	< 0.001
Positive	49 (96)	39 (71)	
Patient age (years)			
≦ 50	20 (39)	17 (31)	P for trend 0.284*
> 50	31 (61)	38 (69)	

P-values were obtained by chi-square test.
*: P for trend was obtained by logistic regression models using histological grade, tumor size and patient age as continuous variables.

Table 2. Associations Between Enhancement Patterns and Histopathologic Diagnosis in 106 Invasive Carcinomas

Pattern Total (n = 106)	Papillotubular carcinoma (%)	Solid-tubular carcinoma (%)	Scirrhous carcinoma (%)	Other (%)
A (n = 51)	18 (35)	2 (4)	26 (51)	5 (10)
B (n = 20)	7 (35)	8 (40)	3 (15)	2 (10)
C (n = 24)	5 (21)	1 (4)	15 (63)	3 (13)
D (n = 11)	5 (45)	1 (9)	4 (36)	1 (9)

P = 0.002 by chi-square test

Detailed classification of rim enhancement, of 106 patients, pattern A (negative for rim enhancement) was seen in 51 (48%), pattern B in 20 (19%), pattern C in 24 (23%) and pattern D in 11 (10%) patients.

The histopathologic diagnosis according to enhancement pattern was significantly different ($p = 0.002$) (Table 2). For pattern C, scirrhous carcinoma was observed at a higher rate of 63% compared with other histopathologic types. For pattern A, scirrhous carcinoma was also observed at a rate of 51%.

The odds ratio (OR) for lymph node metastasis was significantly increased for pattern C com-

Table 3. Odds Ratios and 95% Confidence Intervals for Lymph Node Metastasis According to Enhancement Patterns

Pattern Total (n = 105)	Lymph node metastasis		OR (95%CI)
	Positive (%)	Negative (%)	
A (n = 50)	14 (28)	36 (72)	1 (referent)
B (n = 20)	8 (40)	12 (60)	1.7 (0.6-5.1)
C (n = 24)	15 (63)	9 (38)	4.3 (1.5-12.0)
D (n = 11)	5 (45)	6 (55)	2.1 (0.6-8.2)

OR, Odds ratio; CI, Confidence interval

Table 4. Associations of Enhancement Patterns and Blood Vessel Invasion in 106 Invasive Carcinomas

Pattern Total (n = 106)	Blood vessel invasion	
	Positive (%)	Negative (%)
A (n = 51)	0 (0)	51 (100)
B (n = 20)	1 (5)	19 (95)
C (n = 24)	6 (25)	18 (75)
D (n = 11)	1 (9)	10 (91)

P = 0.001 by Fisher's exact test

pared to pattern A (OR = 4.3, 95% CI: 1.5-12.0) (Table 3). Multiple logistic regression analysis revealed that this association was not affected by the effects of other prognostic factors.

Pattern C accounted for 6 (75%) of 8 cases with blood vessel invasion. There was a significant difference in the frequency of blood vessel invasion among the four groups of enhancement patterns ($p = 0.001$ by Fisher's exact test) (Table 4). When we limited the analysis to cases with rim enhancement (patterns B, C and D), there was no significant difference in the frequency of blood vessel invasion among the three groups with rim enhancement.

The distribution of histological grade according to the enhancement patterns was statistically different ($p = 0.003$). There was no case of grade 1 cancer among patterns B, C, and D which had rim enhancement. More than half the number of each pattern showed grade 2 (Table 5).

The size of the invasive lesion was categorized into 2 groups, ≤20 mm and >20 mm. The OR for the size of invasive lesion was significantly increased in patterns C (OR = 15.7, 95% CI: 3.3-74.1) and B (OR = 4.3, 95% CI: 1.3-13.6) compared with

Table 5. Associations of Enhancement Patterns and Histological Grade in 95 Invasive Ductal Carcinomas

Pattern Total (n = 95)	Histological grade		
	Grade 1 (n = 7) (%)	Grade 2 (n = 66) (%)	Grade 3 (n = 22) (%)
A (n = 46)	7 (15)	35 (76)	4 (9)
B (n = 18)	0 (0)	9 (50)	9 (50)
C (n = 21)	0 (0)	14 (67)	7 (33)
D (n = 10)	0 (0)	8 (80)	2 (20)

P = 0.003 by chi-square test

pattern A (Table 6). The association between pattern C and the tumor size was not affected by the effects of other prognostic factors, using a multiple logistic regression model. The association between pattern B and tumor size was not significant after adjusting for the effect of hormone receptor status (adjusted OR = 3.2, 95% CI: 0.9-10.9).

The cases with rim enhancement tended to be hormone receptor negative. The OR of hormone receptor positivity was significantly lower for patterns B (OR = 0.06, 95% CI: 0.01-0.3) and D (OR = 0.07, 95% CI: 0.01-0.5) compared with pattern A (Table 7).

There was no significant association between the enhancement patterns and patient age by ANOVA (data not shown).

Discussion

Tumor size, histopathologic type, histological grade, lymph node metastasis, vascular invasion, and hormone receptors are known as prognostic factors of breast cancer, and these prognostic factors contribute to clinical management[10].

The relationships between prognostic factors of breast cancer and contrast-enhanced MRI were recently studied[2, 7, 11-17]. Most studies mainly aimed at the degree of enhancement of breast cancers on MRI to explore the prognostic significance[2, 11-17] while a few papers[7, 11, 12] have described relation between rim enhancement and other prognostic factors of breast cancer.

In 1996, Stomper *et al.*[11] examined the associations between spatial and temporal dynamic MRI enhancement features and DNA S-phase percentages (a measure of cellular proliferative activity) of invasive breast carcinomas (n = 17). They found that increased cell proliferation in invasive cancers

Table 6. Associations Between Enhancement Patterns and Size of Invasive Lesion in 106 Invasive Carcinomas

Pattern Total (n = 106)	Size (mm)			OR (95%CI)
	Mean (SD)	≦ 20 (n = 41) (%)	> 20 (n = 65) (%)	
A (n = 51)	20 (2.1)	30 (73)	21 (32)	1 (referent)
B (n = 20)	28 (2.9)	5 (12)	15 (23)	4.3 (1.3-13.6)
C (n = 24)	35 (2.6)	2 (5)	22 (34)	15.7 (3.3-74.1)
D (n = 11)	31 (8.3)	4 (10)	7 (11)	2.5 (0.6-9.6)
	P = 0.002 by ANOVA			

SD, Standard deviation
OR, Odds ratio; CI, Confidence interval

Table 7. Odds Ratios and 95% Confidence Intervals for Hormone Receptor According to Enhancement Patterns

Pattern Total (n = 106)	Hormone receptor		OR (95%CI)
	Positive (%)	Negative (%)	
A (n = 51)	49 (96)	2 (4)	1 (referent)
B (n = 20)	12 (60)	8 (40)	0.06 (0.01-0.3)
C (n = 24)	20 (83)	4 (17)	0.20 (0.03-1.2)
D (n = 11)	7 (64)	4 (36)	0.07 (0.01-0.5)

OR, Odds ratio; CI, Confidence interval

was significantly associated with a rim enhancement pattern. In 1998, Mussurakis *et al.*[7] determined the presence of rim enhancement by processing the region of interest (ROI) or by assessing subjectively by a reader and they found that there were no significant correlation between rim enhancement (either subjectively or quantitatively) and any of the standard histopathological prognostic factors, including tumor size, differentiation, grade, extensive *in situ* component, lymphovascular invasion, multifocality and multicentricity, and axillary node status, in contrast to our results. We determined the presence of rim enhancement visually but not by processing a ROI. Our visual method to determine the presence of rim enhancement was simple, although the ROI methods may be more objective and reproducible than the visual method. Mussurakis *et al.* also reported that the subjective interpretation of rim enhancement was highly specific but less sensitive in terms of distinguishing cancers from benign lesions compared with the ROI method. Although why the results of associations between rim enhancement and prognostic factors differed between their study and ours is unclear, discrepancies may be due to differences in the pulse sequences used to determine rim enhancement and the number of subjects between their study (n = 64) and ours (n = 106). In 2003, Szabó *et al.*[12] examined the correlation between MR characteristics including rim enhancement and the classical pathologic prognostic factors (tumor size, histologic grade and lymph node status) and immunohistochemically detected biomarkers (c-erbB-2, p53, Ki67, and ER) and reported that the rim enhancement pattern, early maximal enhancement, and washout phenomenon were independently associated with higher histologic grade, positive Ki-67, and negative ER. Our results were similar to theirs in terms of the association between the rim enhancement pattern and higher histologic grade and negative ER status.

There have been some papers which discussed the relationship between prognostic factors of breast cancer and the degree of enhancement on MRI. In 1995, Stomper *et al.*[2] reported that time-intensity curves showed no statistically significant correlations with pathologic size, nodal status, or hormone receptor status of invasive carcinomas. In 1997, Burckley *et al.*[13] reported that correlation between initial enhancement and mean microvessel density was higher in node-positive tumors. In the same year, from the same institute, Mussurakis *et al.*[14] studied the association of the enhancement ratios by ROI analysis with tumor size, histopathological grade, the presence of extensive *in situ* component and lymphovascular invasion, multifocal disease, and axillary lymph node status and showed that there was a strong association

between the enhancement ratios and axillary node status or histological grade. On the other hand, Fischer *et al.*[15] reported that tumor size, tumor grading, axillary lymph nodes showed no significant correlation with the degree of enhancement on dynamic MRI. In 1998, Boné *et al.*[16] reported that there was a significant correlation between contrast enhancement of breast cancer on MRI and both tumor angiogenesis and proliferative cellular activity as shown by proliferating cellular nuclear antigen (PCNA) immunoreactivity and no correlation between contrast enhancement and tumor size, lymph node metastasis, mitotic count or inflammatory response. In 2003, Boné *et al.*[17] reported that multivariate analysis for disease-free survival showed that the signal enhancement ratio and tumor size were significant and independent survival predictors.

There have therefore been various results obtained on the associations between contrast enhanced MRI and prognostic factors, and we have not yet reached a definitive conclusion.

Some investigators have studied the causes of rim enhancement of breast cancers[6, 18]. Buadu *et al.*[6] reported that a high microvessel density in the marginal zone of the viable tumor and/or connective tissues may partly account for the rim pattern of enhancement of breast cancers on MRI. The study by Weidner *et al.* showed that the number of microvesels in the areas of most intensive neovascularization in an invasive breast carcinoma may be an independent predictor of metastatic disease either in axillary lymph nodes or at distant sites (or both)[19] and there have been other reports which showed that the microvessels are located more abundantly at the tumor periphery than the tumor center[13, 18]. Buadu *et al.*[6] reported that early rim enhancement with centripetal progression, which corresponds to pattern C in our study, was fairly specific for carcinomas and seen in invasive carcinomas with a high peripheral and a low central microvessel density, and was associated with central fibrosis and/or necrosis (n = 18; 15 with central fibrosis, 2 with fibrosis and necrosis, and 1 with necrosis alone). In our study, the cases showing pattern C also had a tendency to have much fibrosis. Furthermore, it has been reported that the presence of fibrotic foci is a very useful parameter predicting tumor recurrence and initial distant organ metastasis[20, 21] and centrally necrotizing carcinoma is characterized by early systemic metastasis and an accelerated clinical course[22]. These results appear to explain why the rim enhancement, especially in pattern C, was associated with prognostic factors such as lymph node metastasis and tumor size in our study. Buadu *et al.*[6] reported that among the carcinomas showing early rim enhancement with minimal or no centripetal progression, which corresponds to pattern B in our study, central tumor necrosis was a more common feature than central fibrosis and the central microvessel density was low. The carcinomas with delayed rim enhancement which probably resulted from more rapid central washout of contrast medium, pattern D in our study, were seen to have an expansive growth pattern and tended to have a high marginal microvessel density along with a richly vascularized connective tissue pseudocapsule[6]. We did not measure the microvessel density of our cases and could not find obvious histologic features in either pattern B or D. We speculate that functional differences such as permeability of blood capillaries[23] or physical differences such as pressure of the interstitial components[24] also impact enhancement. In our study, the presence of rim enhancement, especially in patterns B or D, resulted in a significantly lower frequency of hormone receptor positivity. Hormone receptor-negative tumors tend to have low response rates to endocrine therapy[10]. It was reported that absence of tumor necrosis was significantly associated with positive ER[25], that necrosis was significantly associated with low ER levels[26] and elastosis was related to the presence of ER[27-31] and PgR[28, 30, 31]. Therefore, absence of tumor necrosis may result in entire enhancement and partly explain why almost all breast cancers without rim enhancement had hormone receptors. Elastosis may also participate in causing "entire" enhancement, although to our knowledge, there was no report about the relation between elastosis and contrast-enhanced MRI. Matsubayashi *et al.*[18] investigated the histologic basics of rim enhancement of breast masses in detail and reported that small cancer nests, a high ratio of peripheral-to-central microvessel density, peripheral vascular endothelial growth factor (VEGF) expression, and a low ratio of peripheral-to-central fibrosis correlated with early rim enhancement while delayed rim enhancement correlated with a high degree of fibrosis and inflammatory changes.

We compared patterns of rim enhancement in breast cancers with prognostic factors retrospec-

tively. To reveal more clearly the associations between the rim enhancement and various prognostic factors, it is necessary for us to perform a prospective study and multivariate studies for disease-free survival. In addition, the mechanism of rim enhancement of breast cancers have to be clarified.

In summary, rim enhancement of breast cancers on MRI significantly associated with lymph node metastasis, blood vessel invasion, histological grade, tumor size and hormone receptors in our present study. Therefore, the assessment of rim enhancement may be useful for preoperative prediction of prognosis of patients with breast cancer. Contrast-enhanced MRI may offer information not only for loco-regional staging but also for tumor biological activity.

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Effects of Neonatally-administered 17β-estradiol on Induction of Mammary Carcinomas by 7, 12-Dimethylbenz[*a*]anthracene in Female Rats

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Abstract. *Various doses of 17β-estradiol (E_2) were administered subcutaneously to inbred female Sprague-Dawley (SD) rats once at birth. At 50 days after birth, rats in all the groups were given 10 mg of 7, 12-dimethylbenz[a]anthracene (DMBA). In the 1000 μg group, the incidence and number of mammary carcinomas were markedly low, while in the 10 μg group, a large number of mammary carcinomas was noted. Corpora lutea were observed in all rats in the control, 0.1, 1, 10 and 100 μg groups at 50 days old; however, no corpora lutea were observed in any rat in the 1000 mg group at age 50 days and at sacrifice. Observation of the whole-mount specimens showed a low number of terminal end buds (TEBs) in the 1000 μg group and a high number in the 10 μg group. It is suggested that neonatal administration of E_2 affects the gonadotropin-secreting system, resulting in a decrease of progesterone, which is thought to influence the progression of mammary carcinomas induced by DMBA. Moreover, neonatal administration of E_2 directly affects the mammary glands, and it is suggested that E_2 may promote differentiation of TEBs resulting in inhibitory effects on the initiation of mammary carcinomas.*

Mammary carcinoma is on the increase worldwide (1-3), and determination of the cause is essential for its prevention. It is known, both epidemiologically and experimentally, that the development of mammary carcinomas is related to sex hormones, and it must be determined whether substances that act as sex hormones affect the development of mammary carcinomas. It is thought that many environmental endocrine disruptors, particularly during the perinatal or neonatal period, act like sex hormones, directly or indirectly disrupting the reproductive function, thereby inhibiting reproduction (4-6). There is a possibility, therefore, that endocrine-disrupting chemicals might be one of the factors involved in this phenomenon.

It has been shown that a high dose of sex hormones affects the development of rat mammary carcinomas induced by the administration of chemical carcinogens when administered during the neonatal period (7-10).

In the present study, various doses of 17β-estradiol were administered to neonatal female rats at a critical period of morphogenesis and functional development of the mammary glands to examine the effect on 7, 12-dimethyl[*a*]anthracene (DMBA)-induced mammary carcinogenesis.

Materials and Methods

The animals were inbred Sprague-Dawley (SD) female rats, maintained in a filtered air laminar flow at the Division of Laboratory Animal Science, Research Center for Life Science Resources, Kagoshima University, Japan. The rats were given a commercial diet (CE-2, CLEA Inc., Tokyo, Japan) and tap water was available *ad libitum*. The room temperature was maintained at 25°C ± 2°C and relative humidity at 55% ± 10%, with a 12 h-light/dark cycle. The use of animals in this research complied with all the relevant guidelines of the Japanese government and Kagoshima University.

17β-estradiol (E_2, Sigma Chemical Co., St. Louis, MD, USA) at 0.1, 1, 10, 100 and 1000 mg, dissolved in 0.05 ml sesame oil was administered subcutaneously to inbred female SD rats once at birth, while in the control group, 0.05 ml sesame oil, was also administered. At 50 days after birth, all rats were given 10 mg of

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7, 12-dimethylbenz[*a*]anthracene (DMBA, Wako Pure Chemical Industries Ltd., Osaka, Japan) dissolved in 1 ml sesame oil by gastric intubation. All the rats, except those sacrificed during the observation period, were examined by palpation to detect mammary tumors until the age of 215 to 238 days. All mammary tumors and organs were fixed in 10% phosphate-buffered formalin, dehydrated and embedded in paraffin wax. The widest cut surface was sectioned to 5 mm, stained routinely with H.E. stain and examined histologically. The mean differences were evaluated by *t*-test.

A number of neonatally-treated rats and intact control rats were necropsied at the age of 50 days and the right abdominal mammary glands were collected. These mammary glands were fixed with 10% buffered formalin for 24 h and then stained with alum carmine for 24 h to prepare whole-mount specimens. Terminal end buds (TEBs) were counted from the distal portions of the mammary gland in whole-mounts examined under a stereoscopic microscope.

Ovaries from rats of age 50 days before administration of DMBA and rats sacrificed at the end of the experimental period were collected and examined microscopically.

The mean differences were evaluated by *t*-test. The incidence of tumors and the percentage of rats with corpora lutea were tested by a 4-fold contingency table.

Results

In the 1000 μg group, significantly low numbers of mammary carcinomas per rat and a low incidence of mammary carcinomas were noted compared with the control group. In the 10 mg group, a significantly high number of mammary carcinomas was noted compared with the control group (Table I).

Rats with corpora lutea in the 0.1, 1, 10 and 100 mg groups showed no significant differences in the incidence of mammary carcinomas compared with the control group, although it was not noted (0%) in the 1000 μg group. Observation of the progression of the mammary carcinomas revealed significantly high numbers of mammary carcinomas per rat in the 10 μg group at 200 and 250 days and in the 100 μg group at 200 days, compared with the control group (Tables II and III).

Rats without corpora lutea in the 0.1, 1, 10 and 100 μg groups showed a significantly high incidence of mammary carcinomas compared with the 1000 μg group. Observation of the progression of mammary carcinomas revealed a small number of mammary carcinomas per rat in the 1000 μg group at 150 and 250 days, compared with the 0.1 μg group (Tables II and IV).

At the end of the experimental period, corpora lutea in the ovaries were noted at 79%, 80%, 74% and 55% in the 0.1, 1, 10 and 100 μg groups, respectively. However, no corpora lutea in the ovaries were observed in the 1000 μg group. Significantly low ovary weights were noted in the 1000 μg group compared with the control group at the end of the experimental period. In rats of groups administered 0.1, 1, 10, 100 and 1000 mg E_2 at birth and necropsied at 50 days old, corpora lutea in the ovaries were observed in all rats at up to the 100 μg group, but not in any rat in the 1000 μg group (Tables V and VI).

Table I. *Effects of various dosage levels of E_2 on incidence and number of mammary carcinomas and days of mass detection.*

Group	Number of rats examined	Incidence of mammary carcinomas	Number of carcinomas /rat Mean±SD	Days of mass detection mammary Mean±SD
Control	21	16 (76%)	1.4±1.2	159.0±70.7
17β-estradiol 0.1 μg/body	14	10 (71%)	2.0±2.2	144.2±66.8
17β-estradiol 1 μg/body	15	13 (87%)	2.3±1.8	173.0±59.6
17β-estradiol 10 μg/body	27	24 (89%)	2.4±1.6[a]	164.7±55.8
17β-estradiol 100 μg/body	20	14 (70%)	2.0±2.3	150.2±59.7
17β-estradiol 1000 μg/body	12	3 (25%)[b]	0.3±0.7[b]	157.0±12.5

[a]$p<0.05$: significantly different from the control group
[b]$p<0.01$: significantly different from the control group

Observation of the whole-mount specimens of mammary glands at 50 days revealed a significantly high number of TEBs in the 10 μg group and a significantly low number in the 1000 μg group compared with the control group (Table VI).

Discussion

In the 0.1, 1, 10, 100, and 1000 μg groups, some rats at up to 100 mg showed no corpora lutea in the ovaries at sacrifice, although corpora lutea in the ovaries were observed in all rats in the 0.1, 1, 10 and 100 μg groups at the age of 50 days. Conversely, no corpora lutea in the ovaries were observed in any rat from the 1000 μg group at the age of 50 days or at sacrifice. Moreover, a significant decrease in ovary weight was noted in the 1000 μg group at sacrifice. According to previous studies, this phenomenon is thought to be due to a disturbance of the gonadotropin-secreting system in the hypothalamus from a high dosage of E_2. A high dosage of testosterone propionate during the neonatal period is converted to estrogen in the hypothalamus, resulting in rats without corpora lutea due to disturbance of the gonadotropin-secreting system. We administered 1.25 mg of

Table II. *Effects of various dosage levels of E_2 on incidence of mammary carcinomas and number of mammary carcinoma per rats with/without corpora lutea.*

Group	Number of rats examined	Rats with mammary carcinoma/rats with corpora lutea	Number of mammary carcinomas/rats with corpora lutea	Rats with mammary carcinoma/rats without corpora lutea	Number of mammary carcinomas/rats without corpora lutea
Control	21	16/21 (76%)	1.4±1.2	–	–
17β-estradiol 0.1 μg/body	14	7/11 (64%)	1.6±1.9	3/3 (100%)[a]	2.7±2.9
17β-estradiol 1 μg/body	15	10/12 (83%)	1.8±1.7	3/3 (100%)[a]	3.0±1.0
17β-estradiol 10 μg/body	27	18/20 (90%)	2.4±1.8	6/7 (86%)[a]	1.6±1.3
17β-estradiol 100 μg/body	20	8/11 (73%)	2.3 ±2.4	7/9 (78%)[a]	1.4±1.0
17β-estradiol 1000 μg/body	12	–	–	3/12 (25%)	0.3±0.7

[a]$p<0.05$: significantly different from the 1000 μg group
–: not detected

Table III. *Effect of various dosage levels of E_2 on number of mammary carcinoma per rats with corpora lutea.*

		Number of rats with mammary carcinomas and corpora lutea Number of mammary carcinomas/rats with corpora lutea				
		Day of mass detection				
Group	Number of rats examined	~50[a]	~100[a]	~150[a]	~200[a]	~250[a]
Control	21	1 (4.8%)	7 (33.3%)	11 (52.4%)	11 (52.4%)	16 (76.2%)
		0.05±0.22	0.5±0.8	0.7±0.8	0.8±0.9	1.4±1.2
17β-estradiol	11	1 (9%)	5 (45%)	6 (55%)	7 (64%)	7 (64%)
0.1 μg/body		0.09±0.30	0.6±0.9	1.4±1.9	1.5±1.9	1.6±1.9
17β-estradiol	12	0 (0%)	2 (17%)	5 (42%)	7 (58%)	10 (83%)
1 μg/body		–	0.4±1.0	0.8±1.2	1.3±1.7	1.8±1.7
17β-estradiol	20	0 (0%)	7 (35%)	14 (70%)	15 (75%)	18 (90%)
10 μg/body		–	0.4±0.6	1.1±1.0	1.8±1.5[b]	2.4±1.8[b]
17β-estradiol	11	0 (0%)	5 (45%)	7 (64%)	8 (73%)	8 (73%)
100 μg/body		–	0.8±1.1	1.5±1.4	1.9±1.6[b]	2.3±2.4
17β-estradiol	0	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
1000 μg/body		–	–	–	–	–

[a]Days after birth (approximately days)
[b]$p<0.05$: significantly different from the control group
–: not detected

testosterone propionate to neonatal rats, and those without corpora lutea in the ovaries were administered 20 mg of DMBA at the age of 50 days and were observed for the development of mammary carcinomas. The results of our previous study showed a significant decrease in the number of induced mammary carcinomas (7, 8). Additional administration of progesterone to these rats showed rapid tumorigenesis of mammary carcinomas (9, 11). From the results, it was considered that the tumorigenesis of mammary carcinomas induced by DMBA was suppressed by a long-term decrease in progesterone during the progression period. Accordingly, one cause of marked suppression of mammary carcinoma induction by E_2 in the 1000 μg group in the present study might have been a decrease in progesterone due to the absence of corpora lutea in the ovaries during the progression period of mammary carcinomas. In all rats dosed at 0.1, 1, 10 and 100 μg of E_2, corpora lutea in the ovaries were observed at the age of 50