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Retinoic Acid Receptor and Retinoid X Receptor in Ductal Carcinoma *in situ* and Intraductal Proliferative Lesions of the Human Breast

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Retinoic acid (RAR) and retinoid X receptors (RXR) are essential in the transcriptional actions of retinoids. To date, RAR and RXR have not been examined in precancerous lesions and/or ductal carcinoma *in situ* (DCIS) in human breast. Therefore, we examined RAR and RXR subtypes in DCIS (58 cases), atypical ductal hyperplasia (ADH) (32 cases), and proliferative disease without atypia (PDWA) (32 cases) to study the status of these RARs and RXRs. Immunoreactivities for RAR α, RXR α, RXR β, and RXR γ were all detected in the nuclei of normal ductal epithelia. Immunoreactivity for RAR β was detected exclusively in the nuclei of myoepithelial cells, but not in normal ductal epithelia. Immunoreactivity for RAR γ was not detected in any of the breast tissues examined except for a few cases of PDWA and ADH, and 11 cases of DCIS. The RXR α labeling index (LI) was significantly higher in both DCIS (mean 77.9) and ADH (mean 77.7) than in PDWA (mean 62.8) ($P<0.001$). RXR β LI was significantly lower in DCIS (mean 81.5) than in both ADH (mean 91.1) and PDWA (mean 91.9) ($P=0.0001$). Immunoreactivity for RAR α, RXR α, RXR β and RXR γ was widely distributed compared to that of RAR β and RAR γ in DCIS, ADH and PDWA. RAR α LI was significantly correlated with Ki67 LI in DCIS ($P=0.0040$), especially in estrogen receptor (ER)-positive DCIS. Our results suggest that RXRs are much more widely distributed than RARs in intraductal proliferative lesions of tne human breast, but ER-positive DCIS cases with high cell proliferative activity are associated with RAR α, suggesting the possible involvement of retinoids through RAR α in tumor cell proliferation in DCIS.

Key words: RAR — RXR — DCIS — Human breast — Proliferative disease

Vitamin A-derived retinoids are well known to regulate cell proliferation and differentiation in a wide range of tissues and cell types.[1, 2] Retinoids can also inhibit the proliferation of a large variety of normal and neoplastic cell types *in vitro*,[3–7] and recently they have been used successfully in the treatment and prevention of a number of human malignant neoplasms, such as acute promyelocytic leukemia.[8, 9] These effects are mainly mediated by two classses of nuclear retinoid receptors, which belong to the steroid/thyroid hormone receptor superfamily, retinoic acid receptors (RARs)[10–14] and retinoid X receptors (RXRs).[15–17] Retinoid receptors are known to function as heterodimers of RAR and RXR, or as RXR homodimers, and to activate transcription in a ligand-dependent manner by binding to retinoic acid-responsive elements (RAREs) located in the promoter region of various target genes.[18] Both RARs and RXRs are composed of three subtypes: α, β, and γ. The expression patterns of these retinoid receptor subtypes have been considered to regulate the expression of distinct target genes and the actions of retinoids.[19]

Despite the established roles of retinoids in the inhibition of growth in human breast cancer cell lines,[20–22] and the potential roles of retinoids in chemoprevention or therapy of breast cancer,[6, 23] relatively limited information is available on the expression of retinoid receptors and/or the actual biological effects of retinoids in human breast carcinoma tissues. In advanced human breast carcinomas, an increased expression of RAR α has been reported,[24] and recently, decreased expression of RAR β mRNA has been reported.[25]

Chemoprevention utilizing retinoids appears to be more effective in the early phase of cancer, or in the premalignant phase than in the advanced phase of cancer.[3, 26, 27] However, expression of retinoid receptors has been little studied in the early phase of breast cancer, i.e., ductal carcinoma *in situ* (DCIS)[28] or other intraductal proliferative lesions such as atypical ductal hyperplasia (ADH). The anti-proliferative effects of retinoids have been recognized mainly in hormone-dependent or estrogen receptor (ER)-positive breast carcinoma, but hardly in hormone-independent or ER-negative breast carcinoma.[29–31] Additionally, it is known that there are more ER-positive cases in low-grade DCIS than in high-grade DCIS, or invasive breast

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carcinoma.[32–34] Therefore, in this study, we examined the expression of RARs and RXRs in both benign human breast tissue and in human breast *in situ* carcinomas. We also examined the correlations among these findings, ER α status, progesterone receptor (PR) status, Ki67 labeling index (LI), c-erbB-2 overexpression, and p53 mutation, in order to further characterize the biological significance of these retinoid receptors in breast carcinoma.

MATERIALS AND METHODS

Cases Surgical pathology specimens were retrieved from the pathology files of Tohoku University Hospital, Sendai, Kawasaki University Hospital, Kurashiki, and Tohoku Kousai Hospital, Sendai. These specimens included 58 cases of DCIS, 32 cases of ADH, and 32 cases of proliferative disease without atypia (PDWA) including moderate and florid hyperplasia of the usual type. Pathological diagnosis was based on the criteria of Dupont and Page[35] and of Ottesen *et al.*[36] Classification of DCIS was based on the Consensus Conference on the Classification of Ductal Carcinoma In Situ in 1997.[37] Non-pathological breast tissues were available for examination in 13, 12 and 12 cases of DCIS, ADH and PDWA, respectively. All of these specimens were fixed in 10% formalin for 24 to 48 h and embedded in paraffin.

Antibodies Polyclonal antibodies for RAR α (sc-551), RAR β (sc-552), RAR γ (sc-550), RXR α (sc-553), and RXR γ (sc-555) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal antibody for RXR β was kindly given to us by Dr. Sugawara (2nd Department of Internal Medicine, Tohoku University, Sendai). The detailed characterization of this antibody has been reported.[38] Antibodies against ER α, PR, P53, and c-erbB-2 and Ki67 antibody (MIB1) were commercially obtained. The source, optimal dilution, and pretreatment method for immunostaining are summarized in Table I.

Immunohistochemistry Serial 3 μm thick sections were prepared. The first and last sections were stained with hematoxylin-eosin for confirmation of the pathological diagnosis. Sections from paraffin formaldehyde-fixed blocks were deparaffinized in xylene and dehydrated in a gradient of ethanol. When necessary, an antigen retrieval method was employed. Intrinsic peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 min at room temperature. Sections were subsequently washed in distilled water and 0.01 mol/liter phosphate-buffered saline (PBS). Sections were then incubated with 1% normal goat serum for the polyclonal antibody, or 1% normal rabbit serum for the monoclonal antibody, in PBS for 30 min at room temperature, followed by an overnight incubation with the primary antibody at 4°C. The dilutions of primary antibodies employed in this study are summarized in Table I. The sections were then incubated with biotinylated goat anti-rabbit IgG for polyclonal primary antibodies, or biotinylated rabbit anti-mouse IgG for monoclonal antibodies (Histofine Kit; Nichirei, Tokyo), and with horseradish peroxidase-conjugated streptoavidin (Nichirei). Sections were developed with 3,3′-diaminobenzidine (DAB) and counterstained with hematoxylin. As a negative control for immunostaining, sections were incubated with 0.01 mol/liter PBS, or normal mouse, or rabbit IgG, instead of primary antibodies. No specific immunoreactivity was detected in these tissue sections. Specificity of immunoreactivity was also confirmed by preabsorption of anti-RAR serum, anti-RXR α, or anti-RXR γ with the respective immunizing peptide (sc-551P, sc-552P, sc-550P, sc-553P, and sc-555P obtained from Santa Cruz Co., Ltd.) for 18 h at 4°C prior to the immunohistochemical procedure. Each antibody was incubated with a five-fold (by weight) excess of the respective immunizing peptide.

Scoring of immunoreactivity For evaluation of Ki67, retinoid receptors and steroid receptors, scoring in proliferative lesions were evaluated independently by two of the

Table I. Summary of Primary Antibodies Employed in This Study

Antibodies	Dilution	Antigen retrieval	Source
ER α (monoclonal)	1:1 (prediluted)	Autoclave[a)]	Immunotech (Marseille, France)
PR (monoclonal)	1:30	Autoclave[a)]	Chemicon (Temecula, CA)
Ki67 (monoclonal)	1:50	Microwave[b)]	Immunotech (Marseille, France)
p53 (monoclonal)	1:40	Microwave[b)]	Biomeda (Foster City, CA)
c-erbB-2 (polyclonal)	1:800	None	Nichirei (Tokyo)
RAR α (polyclonal)	1:500	Autoclave[a)]	Santa Cruz (Santa Cruz, CA)
RAR β (polyclonal)	1:500	Autoclave[a)]	Santa Cruz (Santa Cruz, CA)
RAR γ (polyclonal)	1:500	Autoclave[a)]	Santa Cruz (Santa Cruz, CA)
RXR α (polyclonal)	1:500	Autoclave[a)]	Santa Cruz (Santa Cruz, CA)
RXR β (polyclonal)	1:500	Autoclave[a)]	Sugawara *et al*., 1995
RXR γ (polyclonal)	1:500	Autoclave[a)]	Santa Cruz (Santa Cruz, CA)

a) Autoclaved for 5 min at 120°C in 0.01 mol/liter sodium citrate buffer (pH 6.0).
b) Treated for 7.5 min in 0.01 mol/liter sodium citrate buffer (pH 6.0).

authors (NA and TM) in high-power fields (×400) using standard light microscopy. In each case, 200–500 cells in the lesion were counted, and the percentage of immunopositive cells, i.e. the LI, was determined. Cases with discordant results between observers were simultaneously re-evaluated by the same two authors using double-headed light microscopy. For p53 immunostaining, cases with 5% or more tumor cells positive for p53 nuclear immunoreactivity were designated as positive, and other cases were designated as negative according to the report by Poller *et al*.[39] For evaluation of c-erbB-2, cases were defined as positive only when immunoreactivity was identified on the plasma membrane. Other cases were defined as negative.

Statistical analyses A Kruskal-Wallis test was used for comparison of three or more groups, for continuous variables. Mann-Whitney's *U* test was used in the comparison of two groups with continuous variables. χ^2 test was used in the comparison of calculated data for some categories. The correlation analysis between different parameters with continuous variables was assessed by Spearman's rank-order correlation coefficient. *P* values less than 0.05 were considered significant.

RESULTS

Immunohistochemistry of retinoid receptors Immunoreactivities for RAR α, RXR α, RXR β, and RXR γ were all detected in the nuclei of normal ductal epithelial and myoepithelial cells, but immunoreactivity for RAR α was weak (Fig. 1). Immunoreactivity for RAR β was detected exclusively in the nuclei of myoepithelial cells, but not of normal ductal epithelia. Immunoreactivity for RAR γ was not detected in any of the cases examined except for three cases of PDWA, two cases of ADH and 11 cases of DCIS. The distribution of LI for each subtype of retinoid receptor is summarized in Table II.

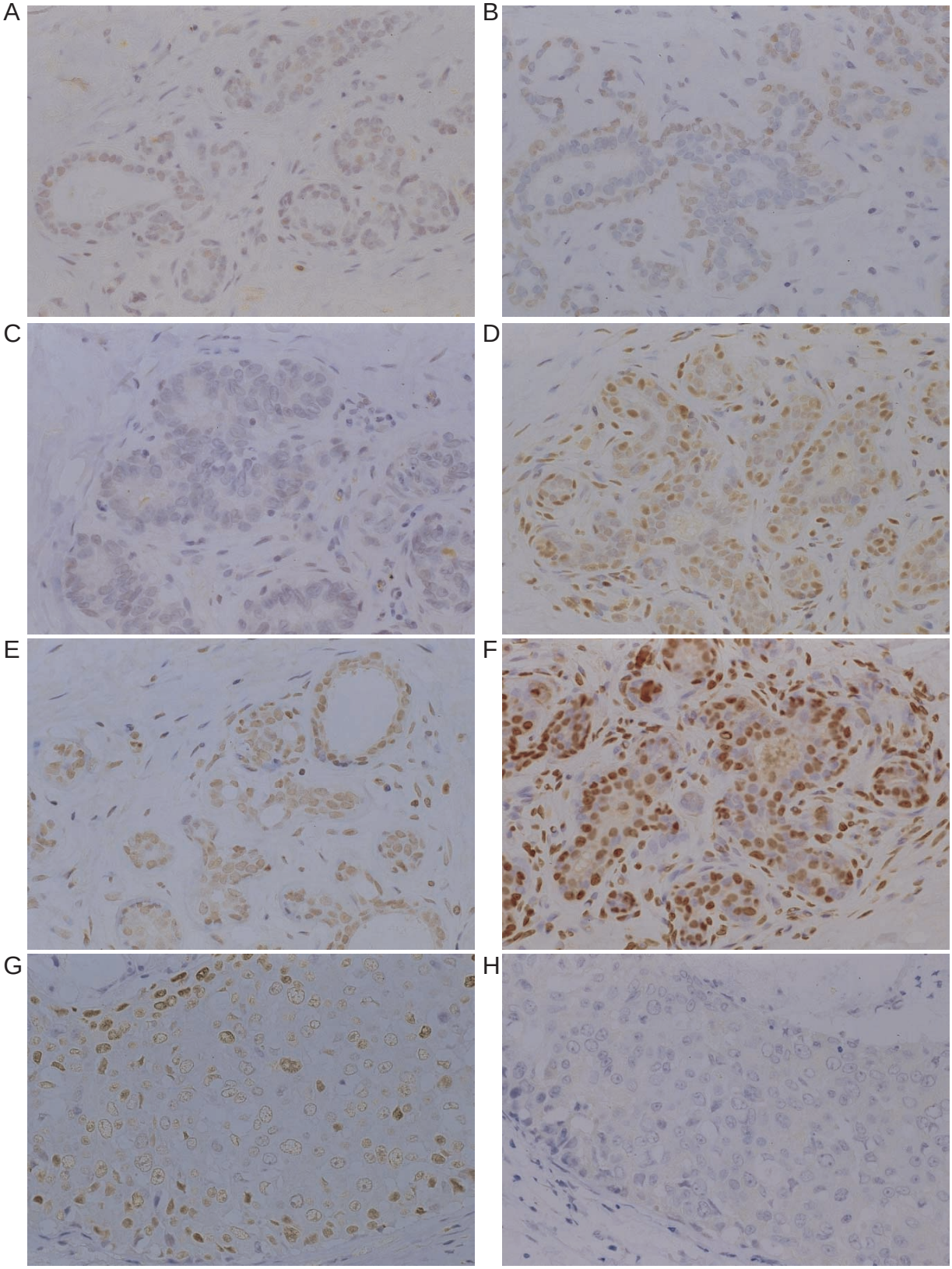
RXR α LI was significantly higher in both DCIS (mean 77.9, 95% confidence interval (CI) 72.9–82.9) and ADH (mean 77.7, 95% CI 72.0–83.4) than in PDWA (mean 62.8, 95% CI 55.1–70.0) (Kruskal-Wallis, $P<0.001$). On the other hand, RXR β LI was significantly lower in DCIS (mean 81.5, 95% CI 77.7–85.3) than in both ADH (mean 91.1, 95% CI 89.1–93.1) and PDWA (mean 91.9, 95% CI 89.3–94.5) (Kruskal-Wallis, $P=0.0001$). There were no other differences in retinoid receptor subtype LIs among PDWA, ADH, and DCIS.

In DCIS, RAR α LI was significantly correlated with Ki67 LI (Spearman's rank test, $P=0.0040$), and this correlation was especially marked in ER α-positive DCIS cases (Fig. 2). There was no such correlation detected in PDWA or ADH cases. There was no significant correlation between Ki67 LI and the LI for any other retinoid receptor in any histological category. RXR α LI was significantly correlated with ER α LI in PDWA and ADH (Spearman's rank test, $P=0.0253$ and $P=0.0331$, respectively). In DCIS, RXR α LI tended to be correlated with ER α LI, but this correlation did not reach statistical significance.

There was no significant correlation between RAR α LI and LI for any of the subtypes of RXR in DCIS cases. Among RXRs, RXR α and RXR β were significantly correlated (Spearman's rank test, $P<0.001$), as were RXR α LI and RXR γ LI (Spearman's rank test, $P<0.01$). Both RXR β LI and RXR γ LI were higher in cases with necrosis than in those without necrosis (Table III). There was no significant correlation between RAR α LI or LI for any subtype of RXR and immunoreactivity for c-erbB-2 or p53 in DCIS (data not shown). On the other hand, there was no significant correlation between RAR α LI and LI for any subtype of RXR in ADH and PDWA cases, and there was also no significant correlation among RXRs (data not shown).

DISCUSSION

Retinoids are potent chemopreventive agents utilized in the treatment regimes for various malignant neoplasms, including breast cancer. Retinoid receptors, RARs and RXRs, in target cells are essential for retinoid action. We studied the expression of these receptors in intraductal proliferative lesions of human breast by immunohistochemistry. Marked variations were detected in the patterns of expression of retinoid receptors, RAR α, RAR β, RAR γ, RXR α, RXR β, and RXR γ, in DCIS and other intraductal proliferative lesions of human breast. Chambon[19] noted that the retinoid receptor subtypes showed specific patterns of expression during embryonic development and within different organs in adults, suggesting that the spatial and temporal expression patterns of retinoid receptor subtypes regulate the expression of distinct genes in various tissues. Therefore, our results suggest that different mechanisms may be involved in the regulation of retinoid receptor expression in these breast disorders. Among retinoid receptor subtypes, immunoreactivity for RAR α, RXR α, RXR β and RXR γ was widely distributed compared to that for RAR β and RAR γ in DCIS and intraductal proliferative lesions, such as PDWA or ADH. Our findings appear to indicate that retinoid actions mediated via RXRs are predominant over those mediated via RARs. In head and neck squamous cell carcinoma cells, retinoid receptors are involved in the growth-inhibitory effects of retinoids, and RXR-RAR heterodimers rather than RXR-RXR homodimers are considered to be the major mediators of growth inhibition by retinoids.[40] In addition, RAR-RXR heterodimers are reported to play an important role in mediating the growth-inhibitory effects of most retinoids in human bronchial epithelial cells.[27] The results of these previously reported studies suggest that RAR-RXR heterodimers are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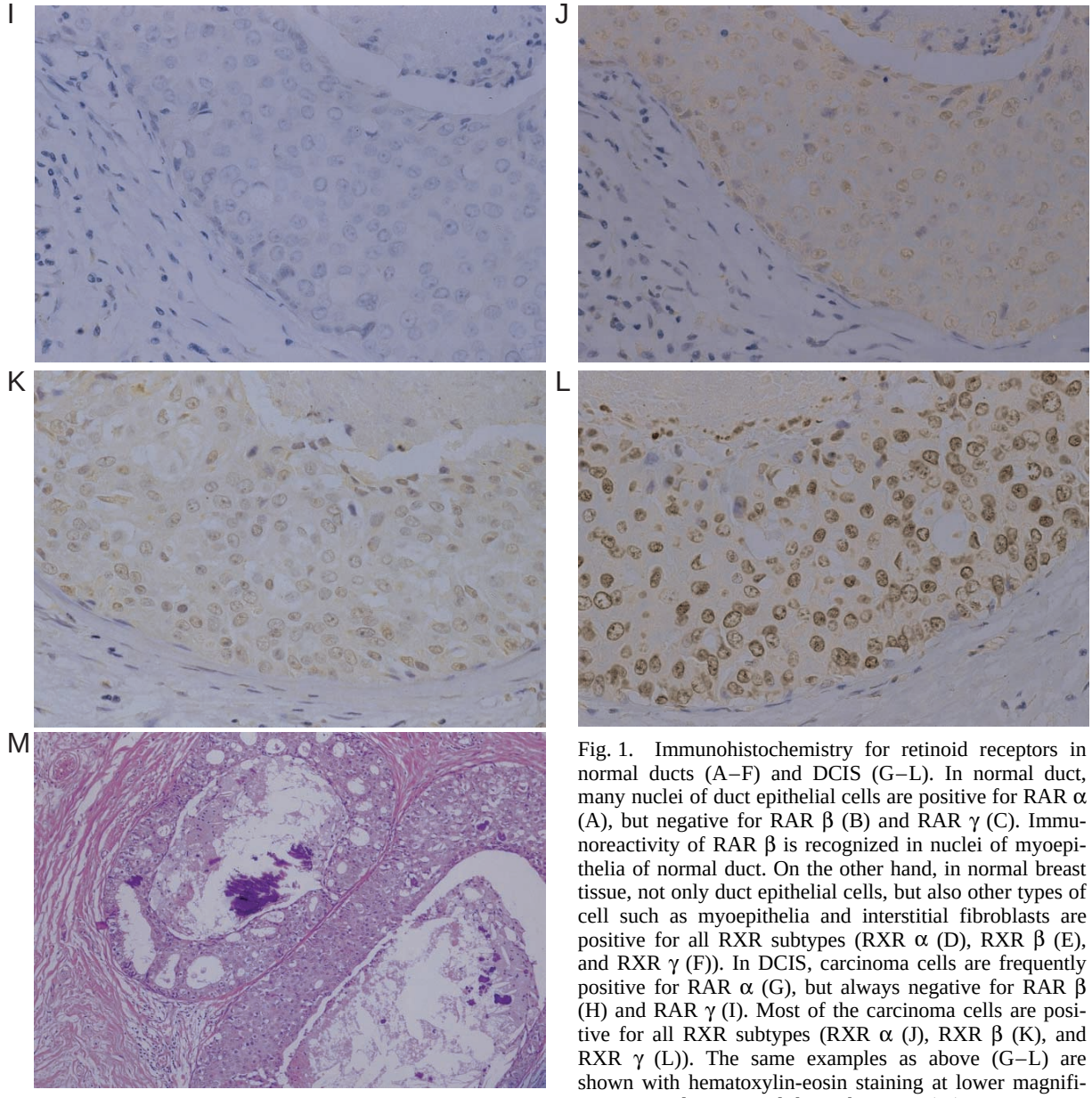

Fig. 1. Immunohistochemistry for retinoid receptors in normal ducts (A–F) and DCIS (G–L). In normal duct, many nuclei of duct epithelial cells are positive for RAR α (A), but negative for RAR β (B) and RAR γ (C). Immunoreactivity of RAR β is recognized in nuclei of myoepithelia of normal duct. On the other hand, in normal breast tissue, not only duct epithelial cells, but also other types of cell such as myoepithelia and interstitial fibroblasts are positive for all RXR subtypes (RXR α (D), RXR β (E), and RXR γ (F)). In DCIS, carcinoma cells are frequently positive for RAR α (G), but always negative for RAR β (H) and RAR γ (I). Most of the carcinoma cells are positive for all RXR subtypes (RXR α (J), RXR β (K), and RXR γ (L)). The same examples as above (G–L) are shown with hematoxylin-eosin staining at lower magnification in order to reveal the architecture (M).

more strongly associated with carcinogenesis than RXR-RXR homodimers. RXRs are therefore more widely distributed than RARs, but alterations of RARs may play more important roles as restrictive factors in carcinogenesis.

Chemoprevention has been frequently used in the management of cancer. Retinoids are considered potent agents for chemoprevention of various malignant neoplasms.[3, 26, 27] Our study demonstrated that RAR α LI was significantly correlated with Ki67 LI in DCIS, especially that of hormone-dependent DCIS, that is, ER-positive DCIS. This observation is compatible with a previous report that indicated a positive correlation between the expression of RAR α and proliferative activity.[24] Cell proliferation of ER-positive breast cancer has been reported to be inhibited by retinoic acid, likely via RAR α.[29–31] These results suggest that, in ER-positive ductal carcinoma with a high proliferative rate, retinoids can inhibit the proliferation of tumor cells through their binding to RAR α. Therefore, retinoids may be of use in the prevention of intraductal

carcinoma of human breast in the high-risk group of patients, such as those diagnosed with ADH.

There are few studies that have investigated the expression of RXRs in human breast cancer, especially in DCIS.[28] RXR α LI was significantly higher in both DCIS and ADH than in PDWA, but RXR β LI was significantly lower in DCIS than in both ADH and PDWA. The differences of LI were relatively small compared to the LIs of RXR α or RXR β in these lesions. However, further investigations are needed to clarify the differences of these RXR subtypes among PDWA, ADH, and DCIS because the function of each RXR subtype in the mammary gland has yet to be clearly defined. In addition, RXR α LI and ER α LI were both significantly correlated in PDWA and ADH, but not in DCIS. Some recent studies have demonstrated an interaction between estrogen action and RXRs/or 9-*cis* retinoic acid and/or peroxisome proliferator-activated receptors.[23, 41, 42] Results from our study are also consistent with those of other investigators, but further

Table II. Summary of Immunohistochemical Character for Each Histological Category and Age of Patients

	PDWA (n=32)	ADH (n=32)	DCIS (n=58)
Age*	43.8 [40.4–47.3]	42.6 [38.5–46.7]	51.2 [48.0–54.4]
p53†	0/32	0/32	7/58
c-erbB-2*	0/32	0/32	14/58
Ki67 LI¶	3.5 [2.3–4.7]	4.3 [3.3–5.4]	9.6 [8.3–10.9]
ER LI¶	33.3 [25.8–40.8]	65.0 [54.8–75.3]	64.7 [55.0–74.5]
PR LI†	26.8 [19.1–34.5]	48.4 [35.3–61.5]	47.3 [37.5–57.1]
RAR α LI	19.4 [11.5–27.4]	27.6 [16.0–39.2]	31.1 [22.5–39.8]
RAR β LI	0.2 [−0.2–0.5]	0.3 [0–0.6]	1.8 [0.5–3.0]
RAR γ LI	1.2 [−0.3–2.7]	0.2 [−0.1–0.4]	1.7 [−0.4–3.8]
RXR α LI*	62.8 [55.6–70.0]	77.7 [72.0–83.4]	77.9 [72.9–82.9]
RXR β LI‡	91.9 [89.3–94.5]	91.1 [89.1–93.1]	81.5 [77.7–85.3]
RXR γ LI	80.9 [50.8–111.1]	83.1 [73.1–93.1]	84.9 [78.8–90.9]

Each value is the mean of all cases examined and values in parentheses are 95% confidence intervals except for p53 and c-erbB-2, for which numbers of positive cases/total cases are indicated. * P<0.001, ¶ P<0.0001, † P<0.05, ‡ P=0.0001.

Table III. Labeling Index for Each Subtype of Retinoid Receptor in Relation to the Presence of Necrosis or the Nuclear Grade in DCIS

	Nuclear grade			Necrosis	
	1	2	3	Present	Absent
RAR α LI	26.7 (n=13)	34.3 (n=36)	24.7 (n=9)	38.3 (n=21)	27.1 (n=37)
RAR β LI	1.8 (n=13)	2.0 (n=34)	2.1 (n=9)	0.5 (n=20)	2.5 (n=36)
RAR γ LI	1.7 (n=13)	0.01 (n=34)	0.02 (n=9)	4.6 (n=20)	0.1 (n=36)
RXR α LI	78.7 (n=13)	79.3 (n=33)	71.6 (n=9)	82.5 (n=19)	75.5 (n=36)
RXR β LI	78.9 (n=11)	82.2 (n=26)	82.8 (n=9)	87.3[a] (n=17)	78.2[a] (n=29)
RXR γ LI	73.3 (n=8)	86.9 (n=25)	93.1 (n=5)	92.6[b] (n=15)	79.8[b] (n=23)

Mean values of labeling indices are shown.
a) P=0.0098.
b) P=0.0066.

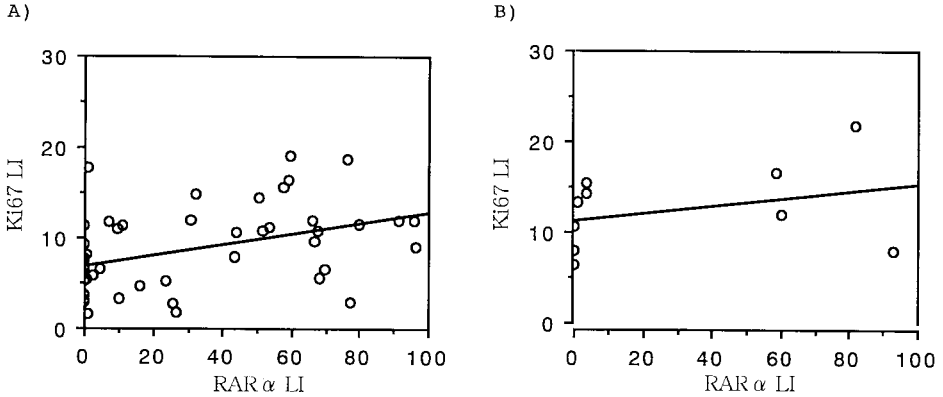

Fig. 2. Correlation between labeling index for RAR α and Ki67 in DCIS (Spearman's rank test). A) ER(+) (n=46). P=0.0049. B) ER(–) (n=10). P=0.1158.

investigations are required to clarify the exact role of retinoid receptor subtypes in breast carcinoma.

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REFERENCES

1) Peto, R., Doll, R., Buckley, J. D. and Sporn, M. B. Can dietary beta-carotene materially reduce human cancer rates? *Nature*, **290**, 201–208 (1981).
2) De Luca, L. M. Retinoids and their receptors in differentiation, embryogenesis, and neoplasia. *FASEB J.*, **5**, 2924–2933 (1991).
3) Hong, W. K., Lippman, S. M., Hittelman, W. N. and Lotan, R. Retinoid chemoprevention of aerodigestive cancer: from basic research to the clinic. *Clin. Cancer Res.*, **1**, 677–686 (1995).
4) Lee, P.-P. H., Lee, M.-T., Darcy, K. M., Shudo, K. and Ip, M. M. Modulation of normal mammary epithelial cell proliferation, morphogenesis, and functional differentiation by retinoids: a comparison of the retinobenzoic acid derivative RE80 with retinoic acid. *Endocrinology*, **136**, 1707–1717 (1995).
5) Seewaldt, V. L., Caldwell, L. E., Johnson, B. S., Swisshelm, K., Collins, S. J. and Tsai, S. Inhibition of retinoic acid receptor function in normal human mammary epithelial cells results in increased cellular proliferation and inhibits the formation of a polarized epithelium *in vitro*. *Exp. Cell Res.*, **236**, 16–28 (1997).
6) Toma, S., Isnardi, L., Riccardi, L. and Bollag, W. Induction of apoptosis in MCF-7 breast carcinoma cell line by RAR and RXR selective retinoids. *Anticancer Res.*, **18**, 935–942 (1998).
7) Weber, E., Ravi, R. K., Knudsen, E. S., Williams, J. R., Dillehay, L. E., Nelkin, B. D., Kalemkerian, G. P., Feramisco, J. R. and Mabry, M. Retinoic acid-mediated growth inhibition of small cell lung cancer cells is associated with reduced myc and increased p27Kip1 expression. *Int. J. Cancer*, **80**, 935–943 (1999).
8) Huang, M.-E., Ye, Y.-C., Chen, S.-R., Chai, J.-R., Lu, J.-X., Zhoa, L., Gu, L.-J. and Wang, Z.-Y. Use of all-*trans* retinoic acid in the treatment of acute promyelocytic leukemia. *Blood*, **724**, 567–572 (1988).
9) Kogan, S. C. and Bishop, J. M. Acute promyelocytic leukemia: from treatment to genetics and back. *Oncogene*, **18**, 5261–5267 (1999).
10) Petkovich, M., Brand, N. J., Krust, A. and Chambon, P. A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature*, **330**, 444–450 (1987).
11) Giguere, V., Ong, E. S., Segui, P. and Evans, R. M. Identification of a receptor for the morphogen retinoic acid. *Nature*, **330**, 624–629 (1987).
12) de Thé, H., Marchio, A., Tiollais, P. and Dejean, A. A novel steroid thyroid hormone receptor-related gene inappropriately expressed in human hepatocellular carcinoma. *Nature*, **330**, 667–670 (1987).
13) Brand, N., Petkovich, M., Krust, A., Chambon, P., de Thé, H., Marchio, A., Tiollais, P. and Dejean, A. Identification of a second human retinoic acid receptor. *Nature*, **332**, 850–853 (1988).
14) Krust, A., Kastner, P., Petkovich, K. M., Zelent, A. and Chambon, P. A third human retinoic acid receptor, hRAR-γ. *Proc. Natl. Acad. Sci. USA*, **86**, 5310–5314 (1989).
15) Hamada, K., Gleason, S. L., Levi, B.-Z., Hirschfeld, S., Appella, E. and Ozato, K. H-2R II BP, a member of the nuclear hormone receptor superfamily that binds to both the regulatory element of major histocompatibility class I genes and the estrogen response element. *Proc. Natl. Acad. Sci. USA*, **86**, 8289–8293 (1989).
16) Mangelsdorf, D. J., Ong, E. S., Dyck, J. A. and Evans, R. M. Nuclear receptor that identifies a novel retinoic acid response pathway. *Nature*, **345**, 224–229 (1990).
17) Mangelsdorf, D. J., Borgmeyer, U., Heyman, R. A., Zhou, J. Y., Ong, E. S. and Oro, A. E. Characterization of three RXR genes that mediate the action of 9-*cis*-retinoic acid. *Genes Dev.*, **6**, 329–344 (1992).
18) Leid, M., Kastner, P. and Chambon, P. Multiplicity generates diversity in the retinoic acid signaling pathways. *Trends Biochem. Sci.*, **17**, 427–433 (1992).
19) Chambon, P. A decade of molecular biology of retinoic acid receptors. *FASEB J.*, **10**, 940–954 (1996).
20) Marth, C., Mayer, I. and Daxenbichler, G. Effect of retinoic acid and 4-hydroxytamoxifen on human breast cancer cell lines. *Biochem. Pharmacol.*, **33**, 2217–2221 (1984).
21) Fontana, J. A., Miranda, D. and Mezu, A. B. Retinoic acid inhibition of human breast carcinoma proliferation is accompanied by inhibition of the synthesis of a M_r 39,000 protein. *Cancer Res.*, **50**, 1977–1982 (1990).
22) van der Burg, B., van der Leede, B. M., Kwakkenbos-Isbrucker, L., Salverda, S., de Laat, S. W. and van der Saag, P. T. Retinoic acid resistance of estradiol-independent breast cancer cell lines coincides with diminished expression of functional retinoic acid receptors. *Mol. Cell. Endo-*

crinol., **91**, 149–157 (1993).
23) Anzano, M. A., Byers, S. W., Smith, J. M., Peer, C. W., Mullen, L. T., Brown, C. C., Roberts, A. B. and Sporn, M. B. Prevention of breast cancer in the rat with 9-*cis*-retinoic acid as a single agent and in combination with tamoxifen. *Cancer Res.*, **54**, 4614–4617 (1994).
24) van der Leede, B. M., Geertzema, J., Vroom, T. M., Dècimo, D., Lutz, Y., van der Saag, P. T. and van der Burg, B. Immunohistochemical analysis of retinoic acid receptor-α in human breast tumors: retinoic acid receptor-α expression correlates with proliferative activity. *Am. J. Pathol.*, **148**, 1905–1914 (1996).
25) Xu, X.-C., Sneige, N., Liu, X., Nandagiri, R., Lee, J. J., Lukmanji, F., Hortobagyi, G., Lippman, S. M., Dhingra, K. and Lotan, R. Progressive decrease in nuclear retinoic acid receptor β messenger RNA level during breast carcinogenesis. *Cancer Res.*, **57**, 4992–4996 (1997).
26) Pastorino, U., Infante, M., Maioli, M., Chiesa, G., Buyse, M., Firket, P., Rosmentz, N., Clerici, M., Soresi, E., Valente, M., Belloni, P. A. and Ravasi, G. Adjuvant treatment of stage I lung cancer with high-dose vitamin A. *J. Clin. Oncol.*, **11**, 1216–1222 (1993).
27) Sun, S.-Y., Kurie, J. M., Yue, P., Dawson, M. I., Shroot, B., Chandraratna, R. A. S., Hong, W. K. and Lotan, R. Differential responses of normal, premalignant, and malignant human bronchial epithelial cells to receptor-selective retinoids. *Clin. Cancer Res.*, **5**, 431–437 (1999).
28) Lawrence, J. A., Merino, M. J., Simpson, J. F., Manrow, R. E., Page, D. L. and Steeg, P. S. A high-risk lesion for invasive breast cancer, ductal carcinoma *in situ*, exhibits frequent overexpression of retinoid X receptor. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 29–35 (1998).
29) Roman, S. D., Ormandy, C. J., Manning, D. L., Blamey, R. W., Nicholson, R. I., Sutherland, R. L. and Clarke, C. L. Estradiol induction of retinoic acid receptors in human breast cancer cells. *Cancer Res.*, **53**, 5940–5945 (1993).
30) Rosenauer, A., Nervi, C., Davison, K., Lamph, W. W., Mader, S. and Miller, W. H., Jr. Estrogen receptor expression activates the transcriptional and growth-inhibitory response to retinoids without enhanced retinoic acid receptor α expression. *Cancer Res.*, **58**, 5110–5116 (1998).
31) Zhu, W.-Y., Jones, C. S., Amin, S., Matsukuma, K., Haque, M., Vuligonda, V., Chandraratna, R. A. S. and De Luca, L. M. Retinoic acid increases tyrosine phosphorylation of focal adhesion kinase and paxillin in MCF-7 human breast cancer cells. *Cancer Res.*, **59**, 85–90 (1999).
32) Hanna, W. M., Kahn, H. J. and Chapman, J.-A. W. The correlation of Ki67 growth factor and ERICA in breast cancer. *Mod. Pathol.*, **5**, 220–223 (1992).
33) Leal, C. B., Schmitt, F. C., Bento, M. J., Maia, N. C. and Lopes, C. S. Ductal carcinoma *in situ* of the breast: histologic categorization and its relationship to ploidy and immunohistochemical expression of hormone receptors, p53, and c-erbB-2 protein. *Cancer*, **75**, 2123–2131 (1995).
34) Moreno, A., Lloveras, B., Figueras, A., Escobedo, A., Ramon, J. M., Sierra, A. and Febra, A. Ductal carcinoma *in situ* of the breast: correlation between histologic classification and biologic markers. *Mod. Pathol.*, **10**, 1088–1092 (1997).
35) Dupont, W. D. and Page, D. L. Risk factors for breast cancer in women with proliferative breast disease. *N. Engl. J. Med.*, **312**, 146–151 (1985).
36) Ottesen, G. L., Graverson, H. P., Blichert-Toft, M., Zedeler, K. and Andersen, J. A. Ductal carcinoma *in situ* of the female breast: short-term results of a prospective nationwide study. *Am. J. Surg. Pathol.*, **16**, 1183–1196 (1992).
37) The Consensus Conference Committee. Consensus conference on the classification of ductal carcinoma *in situ*. *Cancer*, **80**, 1798–1802 (1997).
38) Sugawara, A., Yen, P. M., Qi, Y., Lehcan, R. M. and Chin, W. W. Isoform-specific retinoid-X receptor (RXR) antibodies detect differential expression of RXR proteins in the pituitary gland. *Endocrinology*, **136**, 1766–1774 (1995).
39) Poller, D. N., Roberts, E. C., Bell, J. A., Elston, C. W., Blamey, R. W. and Ellis, I. O. p53 protein expression in mammary ductal carcinoma *in situ*: relationship to immunohistochemical expression of estrogen receptor and c-erbB-2 protein. *Hum. Pathol.*, **24**, 463–468 (1993).
40) Sun, S.-Y., Yue, P., Mao, L., Dawson, M. I., Scroot, B., Lamph, W. W., Heyman, R. A., Chandraratna, R. A. S., Shudo, K., Hong, W. K. and Lotan, R. Identification of receptor-selective retinoids that are potent inhibitors of the growth of human head and neck squamous cell carcinoma cells. *Clin. Cancer Res.*, **6**, 1563–1573 (2000).
41) Keller, H., Givel, F., Perroud, M. and Wahli, W. Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements. *Mol. Endocrinol.*, **9**, 794–804 (1995).
42) Tsibris, J. C., Porter, K. B., Jazayeri, A., Tzimas, G., Nau, H., Huang, H., Kuparadze, K., Porter, G. W., O'Brien, W. F. and Spellacy, W. N. Human uterine leiomyomata express higher levels of peroxisome proliferator-activated receptor γ, retinoid X receptor α, and all-*trans* retinoic acid than myometrium. *Cancer Res.*, **59**, 5737–5744 (1999).