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Advances in Brief

Prevention of Breast Cancer in the Rat with 9-*cis*-Retinoic Acid as a Single Agent and in Combination with Tamoxifen

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Abstract

We show that 9-*cis*-retinoic acid (9*c*RA) is a potent inhibitor of mammary carcinogenesis induced by *N*-nitroso-*N*-methylurea in Sprague-Dawley rats. Rats were first treated with a single dose of *N*-nitroso-*N*-methylurea (50 mg/kg body weight) and then fed non-toxic levels of 9*c*RA (120 or 60 mg/kg of diet). 9*c*RA was highly effective in reducing tumor incidence, average number of tumors per rat, and average tumor burden, as well as extending tumor latency. The combination of 9*c*RA with low levels of tamoxifen (TAM; fed at either 1.0 or 0.5 mg/kg of diet) was particularly effective; addition of 9*c*RA to a TAM regimen doubled the number of animals that were tumor-free at autopsy and significantly diminished tumor number and tumor burden. For suppression of carcinogenesis *in vivo*, 9*c*RA was much more potent than all-*trans*-retinoic acid, both as a single agent or in combination with TAM, although both retinoids had equivalent inhibitory effects on DNA synthesis in cultured human breast cancer cell lines. Both 9*c*RA and all-*trans*-retinoic acid induce the expression of the adhesion molecule, E-cadherin, in the SK-BR-3 cell line. We suggest that clinical evaluation of the combination of 9*c*RA and TAM, either for chemoprevention or for adjuvant therapy, should be considered.

Introduction

The current controversy over the safety and efficacy of the use of tamoxifen to prevent breast cancer in women at high risk emphasizes the need to develop new agents for suppression of carcinogenesis. Since retinoids are useful agents for chemoprevention of cancer (1–3), one candidate for this purpose is 9*c*RA[2] or one of its analogues. The recent findings that 9*c*RA is a high-affinity ligand for both the RAR and RXR families of retinoid receptors (4, 5) and that RXRs form heterodimers with other members of the steroid receptor superfamily (6), focus attention on the critical role of 9*c*RA in retinoid physiology. To date, there have been no reports on the use of 9*c*RA to prevent breast cancer, either in experimental animals or women. We now show that 9*c*RA is highly effective for preventing mammary cancer in the standard rat model which uses a single dose of NMU as carcinogen. Furthermore, we show that 9*c*RA is superior to *at*RA for this purpose and that 9*c*RA enhances the chemopreventive activity of low doses of tamoxifen. Finally, we show that both 9*c*RA and *at*RA can increase expression of the adhesion molecule, E-cad, at cell-cell contact sites in breast cancer cells. Since loss of functional E-cad has been implicated in breast cancer progression (7–13), these results suggest that retinoids may be useful, not only in the prevention of breast cancer, but also in its treatment.

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[2] The abbreviations used are: 9*c*RA, 9-*cis*-retinoic acid; *at*RA, all-*trans*-retinoic acid; NMU, *N*-nitroso-*N*-methylurea; TAM, tamoxifen; E-cad, E-cadherin.

Materials and Methods

Mammary Carcinogenesis Studies. A standard protocol for induction of breast cancer in Sprague-Dawley rats was used (14, 15), with a single i.v. dose of NMU, 50 mg/kg body weight. All palpated tumors were confirmed at autopsy. Retinoids and TAM were incorporated into powdered lab chow as described previously (15) and fed *ad libitum*, beginning 1 week after injection with NMU. 9*c*RA (Kuraray Company, Osaka, Japan) or *at*RA (Hoffmann-La Roche, Nutley, NJ) were fed at either of 2 doses, 120 mg (high dose) or 60 mg (low dose)/kg of diet; TAM (Sigma Chemical, St. Louis, MO) was fed at either 1.0 mg (high dose) or 0.5 mg (low dose)/kg of diet. Methods for statistical analysis have been reported previously (15).

Cell Culture Studies and Immunohistochemistry of E-cad. Human breast cancer cells were cultured as described (15). To measure induction of E-cad, SK-BR-3 cells were treated with either 9*c*RA or *at*RA (10^{-7} M) for 48 h prior to fixation in cold methanol. Immunocytochemical staining of E-cad has been described previously (12) using an antibody (anti-GP-84) directed against the extracellular domain of E-cad.

Results

Inhibition of Mammary Carcinogenesis. In our laboratory, greater than 90% of Sprague-Dawley rats treated with NMU alone (50 mg/kg body weight) develop invasive mammary adenocarcinomas within 3 to 4 months of a single i.v. injection. We report here results obtained on a total of 252 rats treated with NMU, using either 9*c*RA or *at*RA alone or in combination with TAM, to inhibit the development of mammary carcinoma. We chose TAM because of the current ongoing clinical trials being conducted in thousands of women worldwide, evaluating the efficacy of this agent for prevention of breast cancer. At optimal doses, TAM is highly effective in the NMU rat model (16). To evaluate additive or synergistic effects of the combination of either 9*c*RA or *at*RA with TAM, we deliberately used suboptimal doses of TAM.

Two separate experiments were performed, and the results are shown in Table 1 and Fig. 1. In all cases, treatment with chemopreventive agents began 1 week after a single i.v. injection of NMU. The data indicate that 9*c*RA is highly effective in this animal model, and in particular, that it is markedly superior to *at*RA, both as a single agent and in combination with TAM. Chronic feeding of 9*c*RA caused significant suppression of carcinogenesis, as measured by four end points, *i.e.*, tumor incidence, average number of tumors per rat, average tumor burden (the total weight of all of an animal's tumors), and tumor latency. The effect of 9*c*RA on the number of rats that were tumor free at autopsy is particularly striking; in the two experiments (Table 1), only 1 of 48 (2%) of the control rats treated with NMU alone was tumor free, while 24 of 48 (50%; $P < 0.001$) of the rats treated with NMU plus 9*c*RA had no grossly detectable tumors. In addition, statistical analysis indicates that the effects of 9*c*RA on inhibition of tumor number and tumor burden are also highly significant ($P < 0.001$ at the higher dose of 9*c*RA and 0.002 or less at the lower dose of 9*c*RA). Fig. 1*A* also shows that 9*c*RA greatly increases

Table 1 *Prevention of mammary cancer by 9cRA, atRA, TAM, and combinations thereof*

Treatment	Tumor-free (P_1; P_2)	Avg. no. tumors (P_1; P_2)	ATB[a] (P_1; P_2)
	Experiment 1		
Control	0/24	3.6	10.9
9cRA, Hi	9/12 (<0.001)	0.3 (<0.001)	0.8 (<0.001)
9cRA, Lo	5/12 (0.002)	1.6 (0.002)	4.1 (0.002)
atRA, Hi	2/12	2.8	11.2
atRA, Lo	1/12	3.3	12.0
TAM, Lo	3/12 (0.03)	1.8 (0.002)	3.4 (0.004)
9cRA, Hi + TAM, Lo	8/12 (<0.001; 0.05)	0.3 (<0.001; 0.003)	0.5 (<0.001; 0.01)
9cRA, Lo + TAM, Lo	8/12 (<0.001; 0.05)	0.8 (<0.001; <0.04)	2.1 (<0.001; <0.05)
atRA, Hi + TAM, Lo	5/12 (0.002; 0.33)	0.9 (<0.001; 0.05)	2.0 (<0.001; 0.20)
atRA, Lo + TAM, Lo	6/12 (<0.001; 0.20)	1.2 (<0.001; 0.17)	1.3 (<0.001; 0.06)
	Experiment 2		
Control	1/24	2.6	8.5
9cRA, Hi	7/12 (<0.001)	0.6 (<0.001)	3.2 (<0.001)
9cRA, Lo	3/12	2.2	4.8
TAM, Hi	7/12 (<0.001)	0.4 (<0.001)	0.5 (<0.001)
TAM, Lo	6/12 (0.003)	0.8 (0.001)	3.7 (0.01)
9cRA, Hi + TAM, Hi	11/12 (<0.001; 0.08)	0.1 (<0.001; 0.03)	0.07 (<0.001; 0.03)
9cRA, Hi + TAM, Lo	10/12 (<0.001; 0.10)	0.2 (<0.001; 0.02)	0.04 (<0.001; 0.03)
9cRA, Lo + TAM, Hi	10/12 (<0.001; 0.18)	0.3 (<0.001; 0.24)	0.2 (<0.001; 0.20)
9cRA, Lo + TAM, Lo	9/12 (<0.001; 0.20)	0.3 (<0.001; 0.04)	4.9 (0.001; 0.14)

[a] ATB, average tumor burden; the average weight of a rat's tumors at autopsy. In both experiments, rank sum tests were used to compare tumor burdens. P_1, value for the comparison of any chemopreventive treatment *versus* controls treated with NMU alone. P_2, value for the comparison of any combined retinoid + TAM treatment *versus* TAM alone. Doses were as follows: 9cRA or atRA, Hi, 120 mg/kg diet; 9cRA or atRA, Lo, 60 mg/kg diet; TAM, Hi/Lo, 1.0/0.5 mg/kg diet. In Experiment 1, agents were fed for 3 months, and in Experiment 2, agents were fed for 4.5 months. All rats were injected with NMU 1 week before starting diets.

tumor latency. In contrast, only 3 of 24 (13%) of the rats treated with *at*RA (Experiment 1, Table 1) were tumor free at autopsy, and *at*RA had no significant effect on tumor number and tumor burden or on tumor latency (Fig. 1*C*).

In addition to studying its efficacy as a single agent, we also determined whether 9*c*RA would provide additional benefit when combined with TAM. Tables 1 and 2, as well as Fig. 1*B*, show that 9*c*RA adds a statistically significant benefit to a TAM regimen, using the same four end points that were evaluated for single agents. Pooling the results of Experiments 1 and 2 (as shown in Table 2) indicates that the higher dose of 9*c*RA, when added to TAM, doubles the number of rats that are tumor free at autopsy ($P = 0.009$ for the comparison of the combination of 9*c*RA plus TAM against TAM alone), decreases the average number of tumors/rat from 1.3 to 0.2 ($P < 0.001$), and decreases the average tumor burden from 3.5 to 0.3 g ($P = 0.001$), or the 75th percentile of the tumor burden distribution from 5.0 to 0.1 g ($P = 0.001$). Smaller but highly significant effects are also seen for the addition of the lower dose of 9*c*RA to the low dose of TAM. Finally, when all 72 animals treated with the combination of 9*c*RA (at either dose) plus TAM (at either dose) are compared with all 36 animals treated only with TAM alone (at either dose), the *P* values for the efficacy of adding 9*c*RA to TAM are all 0.001 or less, whether one measures effects on inhibition of tumor incidence, inhibition of average number of tumors/rat, or on average tumor burden. None of the chemopreventive agents caused any grossly evident toxicity. Although there was some slightly diminished weight gain in animals fed 9*c*RA, *at*RA, or TAM, the average weights of rats on chemopreventive regimens were always 85% or greater of those fed chow diets alone. Furthermore, since rats fed *at*RA had equivalent growth curves, the beneficial effects of 9*c*RA cannot be ascribed to effects on total caloric intake.

Cell Culture Studies. We analyzed several human breast cancer cell lines to study their response to 9*c*RA compared to *at*RA. As reported by others (17, 18, reviewed in 19), we found that *at*RA inhibited [^{3}H]thymidine incorporation in several differentiated (E-cad-positive) cell lines including MCF-7, T47D, and ZR-75B, while it was essentially inactive in undifferentiated (E-cad-negative) lines such as MDA-231, MDA-453, MDA-436, and BT-549. In the present study, we measured effects of 9*c*RA on the above cell lines and found that 9*c*RA had activity essentially equivalent to that of *at*RA, again depending on the state of differentiation of the particular cell line (data not shown, details to be published elsewhere). However, the growth of one relatively undifferentiated cell line, SK-BR-3, which expressed very low levels of E-cad, was inhibited equivalently by both 9*c*RA and *at*RA. Within 12 h of treatment with either 9*c*RA or *at*RA (10^{-7} M), SK-BR-3 cells exhibited a marked change in morphology (Fig. 2). This morphological change was accompanied by increased expression of E-cad at cell-cell contact sites.

Discussion

We report here, for the first time, the use of 9*c*RA for the prevention of breast cancer in a widely used animal model. In this model, the 9-*cis*-isomer of retinoic acid is clearly superior to the all-*trans*-isomer. The mechanism(s) for this difference need to be determined. Inasmuch as 9*c*RA has high affinity for RXRs as well as RARs (4, 5), broader receptor activation may contribute to the greater activity of 9*c*RA; studies of the extent of conversion of *at*RA to 9*c*RA in mammary epithelium *in vivo* thus need to be performed. However, we could find no significant difference between 9*c*RA and *at*RA in our cell culture studies. Another possibility is that there are significant pharmacokinetic differences between the two isomers, and in particular, that 9*c*RA does not induce its own oxidative inactivation to the same extent as *at*RA. The ability of *at*RA to induce enzymes which lower its own concentration is well known (20, 21) and is presumed to be an important factor in the relapse which occurs during treatment of acute promyelocytic leukemia with *at*RA (21). Thus, further studies on the comparative pharmacokinetics of 9*c*RA and *at*RA are critically needed.

In addition to showing its efficacy as a single agent, we have also found that 9*c*RA markedly enhances the chemopreventive activity of TAM, particularly when TAM is used at very low doses. The highest

dose of TAM given to a rat in the present study is less than 20% of that given to a 60-kg woman receiving the standard 20 mg daily dose (for this calculation, we have assumed that the rat weighs 250 g and eats 10 g of food daily). There is concern at present regarding the safety of chronic administration of the standard 20 mg daily dose of TAM, which is widely used in the adjuvant setting and is currently under study in long-term human chemoprevention trials. The present data suggest that it may be possible to lower the dose of TAM and still retain efficacy if TAM were used in combination with a second agent, such as 9*c*RA. Synergistic interaction of TAM and another retinoid, 4-hydroxyphenyl retinamide, in another model of prevention of breast cancer has been shown previously (22), although the dose of the retinoid was very high and the benefit obtained was not as great as in this study. Furthermore, although we have reported previously that synthetic analogues of vitamin D (deltanoids) can synergize with TAM in the NMU breast cancer model (15), the suppressive effects of this combination were not as great as we have obtained here with 9*c*RA plus TAM.

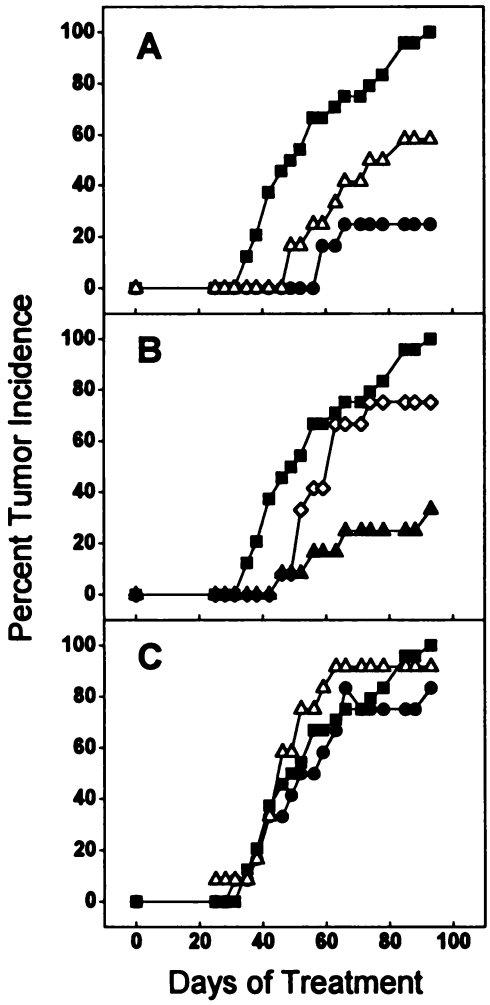

Fig. 1 *A*, NMU was injected into 48 rats, which were randomized into three groups, fed chow diet control (■, 24 rats); 9*c*RA, 120 mg/kg diet (●, 12 rats); or 9*c*RA, 60 mg/kg diet (△, 12 rats), beginning 1 week after NMU. Feeding of 9*c*RA was continued for the remainder of the experiment in the latter two groups. Tumors were palpated twice weekly and confirmed at autopsy. *B*, as in (*A*), (■), 24 rats, control. (◇), 12 rats fed TAM, 0.5 mg/kg diet. (▲), 12 rats fed 9*c*RA, 60 mg/kg diet plus TAM, 0.5 mg/kg diet. Latency curve for 9*c*RA alone is shown in *A*. *C*, as in (*A*), (■), 24 rats, control. (●), 12 rats fed *at*RA, 120 mg/kg diet; (△), 12 rats fed *at*RA, 60 mg/kg diet.

Table 2 *Benefit of addition of 9cRA to TAM in prevention of mammary cancer*

Treatment	Tumor-free (*P*)[a]	Avg. no. (*P*)	Top quartile TB[b], g (*P*)
TAM, Lo	9/24	1.3	5.0
TAM, Lo + 9cRA, Hi	18/24 (0.009)	0.2 (0.001)	0.1 (0.001)
TAM, Lo + 9cRA, Lo	17/24 (0.021)	0.5 (0.01)	0.2 (0.023)

[a] *P*, comparison of the effects of adding 9*c*RA to TAM, when compared to TAM alone.
[b] Top quartile tumor burden is the 75th percentile of the tumor burden distribution for animals in each particular cohort. The value for the top quartile TB in the control group which received no chemopreventive agent was 17.1 g. Rank sum tests were used to compare tumor burdens. Doses of agents as in Table 1.

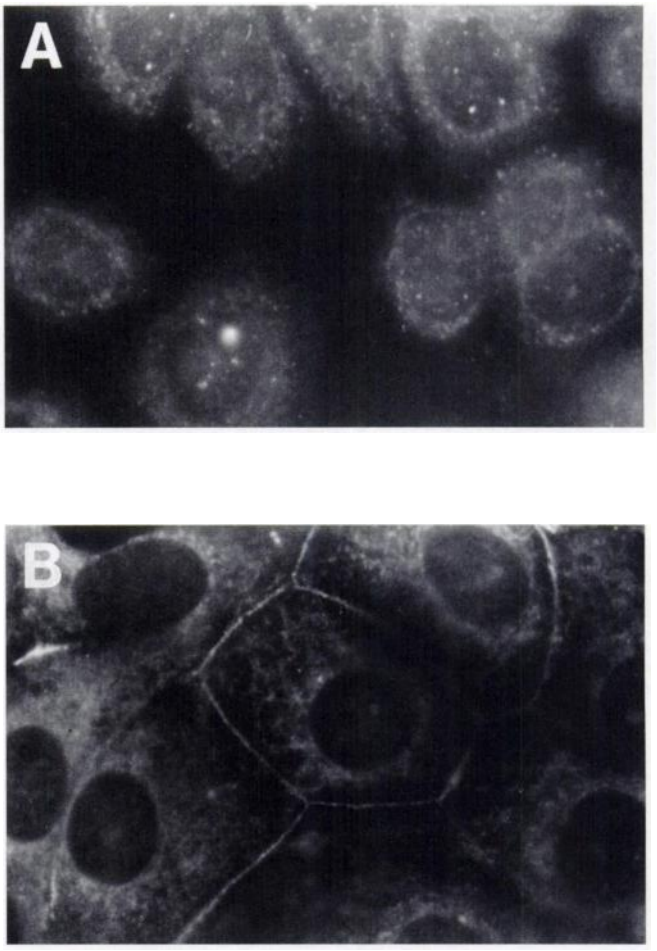

Fig. 2 Immunofluorescent staining of E-cad in SK-BR-3 cells following 48 h of treatment with vehicle alone (*A*) or 10^{-7} M 9*c*RA (*B*).

The present data emphasize the importance of obtaining new information about the safety of chronic administration of low doses of 9*c*RA to human populations at high risk for development of cancer so that it may be clinically evaluated for suppression of carcinogenesis. 9*c*RA is currently in Phase I human trials as a therapeutic agent.[3] Most notably, the striking effects of the combination of two agents, both given at relatively low doses, on inhibition of carcinogenesis indicate that it will be increasingly important to consider the use of combination chemoprevention (23) in the clinical setting of cancer prevention. Although we have emphasized the use of 9*c*RA for chemoprevention in the present study, retinoids also inhibit the growth of many overtly malignant breast cancer cells (17–19). Taken together with the ability of 9*c*RA to increase expression of E-cad, an adhesion molecule often lost in undifferentiated breast carcinomas, the present study suggests testing the addition of 9*c*RA to the standard TAM regimen that is currently in widespread use for adjuvant chemotherapy for postsurgical treatment of breast cancer in women, especially in a subset that is

[3] W. K. Hong, personal communication.

known to be at particularly high risk for relapse. The next problem in the laboratory will be to find a third, or possibly even a fourth, agent to add to this already promising combination regimen to approach the ultimate asymptote of total suppression of mammary carcinogenesis.

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References

1. Sporn, M. B., and Roberts, A. B. Role of retinoids in differentiation and carcinogenesis. Cancer Res., *43:* 3034–3040, 1983.
2. Moon, R. C., Mehta, R. G., and Rao, K. V. N. Retinoids and cancer in experimental animals. *In:* M. B. Sporn, A. B. Roberts, and D. S. Goodman (eds.), The Retinoids: Biology, Chemistry, and Medicine, Ed. 2, pp. 573–595, New York: Raven Press, 1994.
3. Hong, W. K., and Itri, L. M. Retinoids and human cancer. *In:* M. B. Sporn, A. B. Roberts, and D. S. Goodman (eds.), The Retinoids: Biology, Chemistry, and Medicine, Ed. 2, pp. 597–630, New York: Raven Press, 1994.
4. Allegretto, E. A., McClurg, M. R., Lazarchik, S. B., Clemm, D. L., Kerner, S. A., Elgort, M. G., Boehm, M. F., White, S. K., Pike, J. W., and Heyman, R. A. Transactivation properties of retinoic acid and retinoid X receptors in mammalian cells and yeast. J. Biol. Chem., *268:* 26625–26633, 1993.
5. Allenby, G., Janocha, R., Kazmer, S., Speck, J., Grippo, J. F., and Levin, A. A. Binding of 9-*cis*-retinoic acid and all-*trans*-retinoic acid to retinoic acid receptors α, β, and γ. J. Biol. Chem., *269:* 16689–16695, 1994.
6. Mangelsdorf, D. J., Umesono, K., and Evans, R. M. The retinoid receptors. *In:* M. B. Sporn, A. B. Roberts, and D. S. Goodman (eds.), The Retinoids: Biology, Chemistry, and Medicine, Ed. 2, pp. 319–349, New York: Raven Press, 1994.
7. Oka, H., Shiozaki, H., Kobayashi, K., Inoue, M., Tahara, H., Kobayashi, T., Takatsuka, Y., Matsuyoshi, N., Hirano, S., Takeichi, M., and Mori, T. Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. Cancer Res., *53:* 1696–1701, 1993.
8. Gamallo, C., Palacios, J., Suarez, A., Pizarro, A., Navarro, P., Quintanilla, M., and Cano, A. Correlation of E-cadherin expression with differentiation grade and histological type in breast carcinoma. Am. J. Pathol., *142:* 987–993, 1993.
9. Rasbridge, S. A., Gillett, C. E., Sampson, S. A., Walsh, F. S., and Millis, R. R. Epithelial (E-) and placental (P-) cadherin cell adhesion molecule expression in breast carcinoma. J. Pathol., *169:* 245–250, 1993.
10. Frixen, U. H., Behrens, J., Sachs, M., Eberle, G., Voss, B., Warda, A., Lochner, D., and Birchmeier, W. E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J. Cell Biol., *113:* 173–185, 1991.
11. Vleminckx, K., Vakaet, L., Mareel, M. M., Fiers, W., and van Roy, F. M. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. Cell, *66:* 107–119, 1991.
12. Sommers, C. L., Thompson, E. W., Torri, J. A., Kemler, R., Gelmann, E. P., and Byers, S. W. Cell adhesion molecule uvomorulin expression in human breast cancer cell lines: relationship to morphology and invasive capacities. Cell Growth & Differ., *2:* 365–372, 1991.
13. D'souza, B., and Taylor-Papadimitriou, J. Overexpression of c-*erbB*-2 in human mammary epithelial cells signals inhibition of transcription of the E-cadherin gene. Proc. Natl. Acad. Sci. USA, *91:* 7202–7206, 1994.
14. Moon, R. C., Thompson, H. J., Becci, P. J., Grubbs, C. J., Gander, R. J., Newton, D. L., Smith, J. M., Phillips, S. L., Henderson, W. R., Mullen, L. T., Brown, C. C., and Sporn, M. B. *N*-(4-Hydroxyphenyl)retinamide, a new retinoid for prevention of breast cancer in the rat. Cancer Res., *39:* 1339–1346, 1979.
15. Anzano, M. A., Smith, J. M., Uskoković, M. R., Peer, C. W., Mullen, L. T., Letterio, J. J., Welsh, M. C., Shrader, M. W., Logsdon, D. L., Driver, C. L., Brown, C. C., Roberts, A. B., and Sporn, M. B. 1α,25-Dihydroxy-16-ene-23-yne-26,27-hexafluorocholecalciferol (Ro24-5531), a new deltanoid (vitamin D analogue) for prevention of breast cancer in the rat. Cancer Res., *54:* 1653–1656, 1994.
16. Gottardis, M. M., and Jordan, V. C. Antitumor actions of keoxifene and tamoxifen in the *N*-nitrosomethylurea-induced rat mammary carcinoma model. Cancer Res., *47:* 4020–4024, 1987.
17. Lacroix, A., and Lippman, M. E. Binding of retinoids to human breast cancer cell lines and their effects on cell growth. J. Clin. Invest, *65:* 586–591, 1980.
18. Fontana, J. A., Miksis, G., Miranda, D. M., and Durham, J. P. Inhibition of human mammary carcinoma cell proliferation by retinoids and intracellular cAMP-elevating compounds. J. Natl. Cancer Inst., *78:* 1107–1112, 1987.
19. Gudas, L. J., Sporn, M. B., and Roberts, A. B. Cellular biology and biochemistry of the retinoids. *In:* M. B. Sporn, A. B. Roberts, and D. S. Goodman (eds.), The Retinoids: Biology, Chemistry, and Medicine, Ed. 2, pp. 443–520. New York: Raven Press, 1994.
20. Roberts, A. B., Frolik, C. A., Nichols, M. D., and Sporn, M. B. Retinoid-dependent induction of the *in vivo* and *in vitro* metabolism of retinoic acid in tissues of the vitamin A-deficient hamster. J. Biol. Chem., *254:* 6303–6309, 1979.
21. Muindi, J., Frankel, S. R., Miller, W. H., Jakubowski, A., Scheinberg, D. A., Young, C. W., Dmitrovsky, E., and Warrell, R. P. Induction of acquired "resistance" to all-*trans*-retinoic acid in patients with acute promyelocytic leukemia. Blood, *79:* 299–303, 1992.
22. Ratko, T. A., Detrisac, C. J., Dinger, N. M., Thomas, C. F., Kelloff, G. J., and Moon, R. C. Chemopreventive efficacy of combined retinoid and tamoxifen treatment following surgical excision of a primary mammary cancer in female rats. Cancer Res., *49:* 4472–4476, 1989.
23. Sporn, M. B. Combination chemoprevention of cancer. Nature (Lond.), *287:* 107–108, 1980.

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