

Decreased Expression of Retinoic Acid Receptors, Transforming Growth Factor β , Involucrin, and Cornifin in Cervical Intraepithelial Neoplasia¹

Xiao-Chun Xu,² Michele F. Mitchell, Elvio Silva, Anton Jetten, and Reuben Lotan

Departments of Clinical Cancer Prevention [X-C. X.], Gynecologic Oncology [M. F. M.], Pathology [E. S.], and Thoracic/Head and Neck Medical Oncology [R. L.], The University of Texas, M. D. Anderson Cancer Center, Houston, Texas 77030, and Laboratory of Pulmonary Pathobiology, National Institutes of Environmental Health Sciences, Research Triangle Park, North Carolina 27709 [A. J.]

ABSTRACT

Cervical intraepithelial neoplasia (CIN) I, II, and III represent a spectrum of premalignant epithelial changes and are ideal targets for application of chemoprevention strategies. Intermediate end point biomarkers are increasingly being used as surrogate end points to monitor clinical chemoprevention trials. To identify potential biomarkers in cervical epithelium, we analyzed the expression of nuclear retinoic acid receptor (RAR) mRNA by *in situ* hybridization, involucrin, cornifin, and transforming growth factors (TGFs) β 1 and β 2 by immunohistochemistry in cervical specimens, which contained adjacent normal epithelium and CIN lesions from 52 patients. These biomarkers were expressed in all adjacent normal cervical epithelia, whereas all CIN lesions including CIN I, CIN II, and CIN III exhibited decreased expression of RAR- α by 55.8%, RAR- β by 64.7%, RAR- γ by 54.9%, involucrin by 80.8%, cornifin by 88.5%, TGF- β 1 by 89.7%, and TGF- β 2 by 85.7%. Viewed as a whole, these biomarkers were down-regulated in 100% of the CIN lesions. Because all of these biomarkers can be modulated *in vitro* by retinoids, they may serve as intermediate biomarkers for retinoid chemoprevention trials in the patients with CIN lesions.

INTRODUCTION

Cervical cancer, the second most common malignancy in women, remains a prevalent health problem for women worldwide (1, 2). Despite the decades-long decline of age-adjusted

death rates, especially in the United States due to a better understanding of epidemiological risk, screening by the Papanicolaou smear, and improvements in treatment, it is estimated that 12,800 new cases of invasive cervical cancer will be diagnosed in the United States, and 4,800 patients will still die of this disease in 1999 (3). It is, therefore, necessary to continue searching for novel approaches to combat cervical cancer. One of these approaches is chemoprevention.

Chemoprevention is an intervention in the carcinogenesis process by using chemical agents to prevent or delay the progression to cancer (4, 5). CIN³ exhibits various histological abnormalities including nuclear atypia, loss of polarity, presence of abnormal mitoses, or lack of differentiation in cervical epithelium, and this lesion has been defined as a precursor of invasive cervical cancer (6). The lesion is also called squamous intraepithelial lesion in the Bethesda classification (reviewed in Ref. 7). In the present study, our goal is to look at progressive changes as CINs progress to cancer; thus, we use the CIN terminology subsequently. Empirical evidence from published and preliminary clinical reports suggest that retinoids, structural and functional analogues of vitamin A, are effective chemopreventive agents for CINs *in vitro* and *in vivo* (8–18).

Retinoids exert their biological effects by binding to specific nuclear retinoid receptors, which are members of the steroid hormone gene superfamily and ligand-activated, DNA-binding, *trans*-acting, transcription-modulating proteins. The nuclear retinoid receptors are divided into RARs and retinoid X receptors, each of which includes three subtypes, α , β , and γ (19, 20). RARs and retinoid X receptors form either homodimers or heterodimers that bind to a specific DNA sequence, the RA response element, in the promoter regions of genes to modify the expression of target genes (19). Several retinoid-regulated genes carrying these response elements in their regulatory regions have been identified. Each subtype of nuclear retinoid receptor is thought to regulate the expression of distinct genes, because the subtypes exhibit specific patterns of expression during embryonal development and different distributions in adult tissues (19).

For clinical chemoprevention trials, intermediate biomarkers are increasingly being used as surrogate end points to monitor drug efficacy for cancer incidence (21–23). Such biomarkers should be differentially expressed in normal tissue, the premalignant lesion, and cancer, and most important, they must be modulated by the chemopreventive agents (21–23). Various studies have indicated the relevance of quantitative histology,

Received 11/19/98; revised 2/10/99; accepted 3/1/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was partially supported by National Cancer Institute contract NO1-CN-25433-01.

² To whom requests for reprints should be addressed, at Department of Clinical Cancer Prevention, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Box 236, Houston, TX 77030. Phone: (713) 745-2940; Fax: (713) 792-0628; E-mail: xxu@notes.mdacc.tmc.edu.

³ The abbreviations used are: CIN, cervical intraepithelial neoplasia; RA, retinoic acid; RAR, RA receptor; TGF, transforming growth factor; HPV, human papillomavirus.

biological measures of proliferation, differentiation, and genetic instability as markers for cervical chemoprevention trials (6, 23). Our previous reports demonstrated that RAR- β might be useful as an intermediate biomarker for head and neck carcinogenesis and chemoprevention of premalignant oral lesions (24–26). To identify new biomarkers for cervical cancer, we analyzed expression of RARs, as well as several differentiation markers (*e.g.*, involucrin and cornifin), and growth factors (TGF- β 1 and TGF- β 2) in adjacent normal tissues and CIN lesions.

MATERIALS AND METHODS

Surgical Specimens. Tissue samples were obtained from the Department of Pathology at the University of Texas M. D. Anderson Cancer Center (Houston, TX) from cervicectomy for preventive care. The patients had not undergone any previous treatment. These samples were routinely fixed in 10% buffered formalin and embedded in paraffin. All of the specimens were cut into 4- μ m-thick sections and stained with H&E for classification. According to our Institutional Review Board protocols, samples were selected based on histological diagnosis and reviewed by two gynecological pathologists to ensure that both CIN and adjacent normal tissue were present. The pathologist E. S. reviewed all of the slides prepared for biomarker assessment and matched the relevant areas for the measurement.

In Situ Hybridization. A method described previously for nonradioactive *in situ* hybridization using digoxigenin-labeled antisense riboprobes was used to analyze nuclear retinoid receptors in formalin-fixed, paraffin-embedded histological sections (27). The quality and specificity of the digoxigenin-labeled probes were determined by Northern blotting, and the specificity of the binding of antisense riboprobes was verified by using sense probes as controls (24, 27).

Antibodies. Antibodies against TGF- β 1 and TGF- β 2 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Rabbit-anti-involucrin polyclonal antibody was obtained from Biomedical Technologies, Inc. (Stoughton, MA). Rabbit-anti-cornifin polyclonal antibody was produced by Dr. Anton Jetten (National Institutes of Environmental Health Sciences).

Immunohistochemistry. The immunohistochemical localization of involucrin, cornifin, TGF- β 1, and TGF- β 2 was performed by a modified ABC technique used by us previously (28). Control sections were incubated either with normal mouse IgG instead of primary antibodies or with second antibody only. These controls were not stained.

Review and Scoring of the Sections. The stained sections were reviewed and scored by five investigators, including two pathologists using a Nikon multiheaded microscope. The staining of CIN lesion was always compared with staining of adjacent normal tissue to assign to one of the following criteria: less than, greater than, or equal to normal tissue. For statistical analysis, the McNemar test was performed by using STATISTICS version 3.0a (StatSoft, Tulsa, OK) to determine the association between matched pairs of normal epithelia and CIN lesions.

RESULTS

Surgical specimens from 52 patients who underwent core biopsy for preventive care at the University of Texas M. D. Anderson Cancer Center were used in this study. All of the samples contained both adjacent normal tissue and CIN lesion. Specimens from all 52 cases contained normal epithelium, 19 contained CIN I, 28 contained CIN II, 38 contained CIN III lesions, and 6 contained carcinoma *in situ*. To analyze the biomarker expression in CIN lesion, we chose the more severe CIN lesion to be scored for that case if more than one category of CIN lesions existed within the case. Consecutive sections of these samples were analyzed for the expression of RARs, differentiation markers, and TGFs. Fig. 1 shows one example of consecutive tissue sections, which were hybridized with antisense RARs. The positive signal was located in the cytoplasm. The sense probes showed no binding (data not shown here). RAR- α showed no change in CIN I and only a slight reduction in CIN III, whereas RAR- γ showed no change in CIN I, but was completely lost in CIN III. In contrast, RAR- β showed decreased expression already in CIN I lesions and further reduction in CIN III.

The results of the analysis of all specimens are summarized in Table 1. The RARs were expressed in all adjacent normal cervical epithelia, but their expression was reduced in the lesions, including CIN I, CIN II, and CIN III for RAR- α in 55.8%, RAR- β in 64.7%, and RAR- γ in 54.9% of these cases.

Fig. 2 shows adjacent consecutive tissue sections that were stained with involucrin, cornifin, TGF- β 1, and TGF- β 2 by immunohistochemistry. The positive signal was seen in adjacent normal tissues but dramatically decreased or disappeared in CIN lesions for all these markers analyzed. Table 1 shows that the levels of differentiation markers and TGFs were lower than in normal tissues for involucrin in 80.8%, cornifin in 88.5%, TGF- β 1 in 89.7%, and TGF- β 2 in 85.7% of the cases. In comparison with the adjacent normal epithelium, the decreased expression of these biomarkers in CIN lesions was statistically significant (see Table 1). Taken as a whole, the biomarkers were down-regulated in 100% of CIN lesions.

The expression of these biomarkers was reduced in CIN lesions compared with adjacent normal epithelium, but CIN lesions were unnecessary to lose their expression completely; oftentimes, positive cells still existed in the CIN lesions (see RAR- β expression in Fig. 1)

DISCUSSION

Retinoids can inhibit the growth of cervical cancer cells both *in vitro* and *in vivo*, and they are also able to reverse CIN I and CIN II lesions (8–10, 14, 17, 29). In an animal model, vitamin A deficiency produces a series of abnormalities in keratin expression in the cervical epithelia (30), and this might explain the needs of retinoid for maintenance of normal differentiation in cervical epithelium. These findings suggest that the physiological level of RA may play an important role in suppression of carcinogenesis of human cells *in vivo*.

In the present study, we analyzed for first time the expression of RARs and cornifin in normal and premalignant cervical epithelial tissues. In addition, we also analyzed involucrin, TGF- β 1, and TGF- β 2 in the same specimens. These biomarkers were

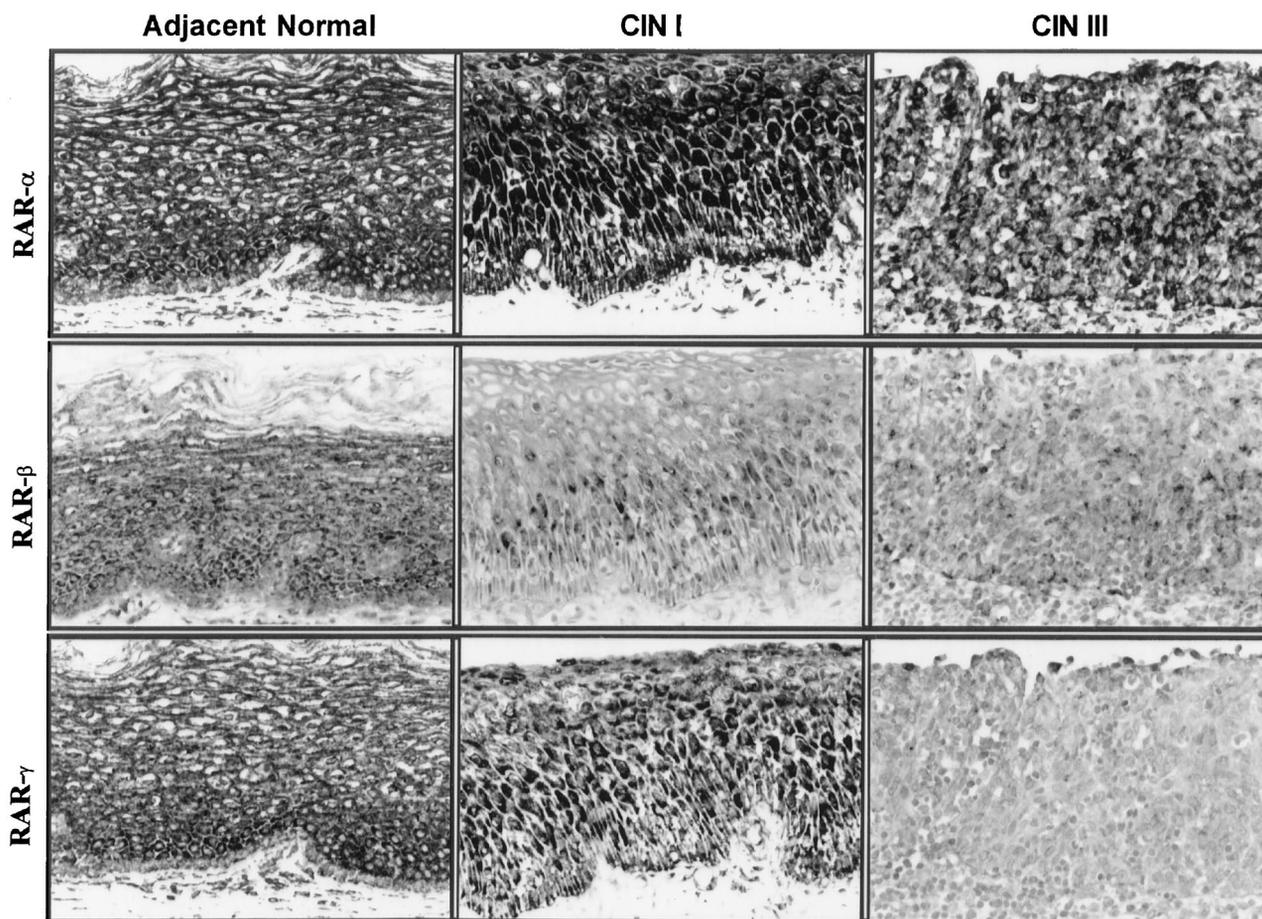


Fig. 1 Localization of RAR mRNAs in surgical specimens from core biopsy by *in situ* hybridization. Consecutive sections of formalin-fixed and paraffin-embedded CIN and its adjacent normal tissue within the same sample were hybridized with RAR- α , RAR- β , or RAR- γ antisense by using digoxigenin-labeled cRNA probes, which results in a positively stained signal located in the cytoplasm.

Table 1 Expression of the biomarkers in adjacent normal epithelia and CIN lesions

Markers	% (positive/total) ^a		
	N > CIN	N = CIN	N < CIN
RAR- α	55.8 (29/52) ^b	32.7 (17/52)	11.5 (6/52)
RAR- β	64.7 (33/51) ^c	27.5 (14/51)	7.8 (4/51)
RAR- γ	54.9 (28/51) ^d	29.4 (15/51)	15.7 (8/51)
Involucrin	80.8 (42/52) ^c	17.3 (9/52)	1.9 (1/52)
Cornifin	88.5 (46/52) ^c	5.8 (3/52)	5.8 (3/52)
TGF- β 1	89.7 (26/29) ^c	10.3 (3/29)	0 (0/29)
TGF- β 2	85.7 (24/28) ^c	14.2 (4/28)	0 (0/28)

^a N, normal epithelium; CIN, including CIN I, II, and III lesions.

^b McNemar test $P < 0.0002$ between normal epithelium and CIN.

^c McNemar test $P < 0.00001$ between normal epithelium and CIN.

^d McNemar test $P < 0.0016$ between normal epithelium and CIN.

all expressed in the adjacent normal epithelium, whereas their levels decreased in the premalignant lesions, including CIN I, CIN II, and CIN III. RARs and TGF- β can be up-regulated by retinoids *in vitro* and *in vivo* (11, 31–33). Except for involucrin and cornifin, which are usually down-regulated by retinoids,

these proteins may be useful as biomarkers of intermediate end points for clinical retinoid chemoprevention trials in cervical cancer. Although the analysis is only semiquantitative, it is quite reliable because the cervical specimens contained both normal epithelium and CIN lesion(s) in the same section; therefore, the comparison of expression levels of these biomarkers is accurate.

Altered expression of nuclear retinoid receptors may lead to carcinogenesis (24, 25, 34). Both ectocervical and endocervical epithelia have been reported to express RARs (35), and altered expression of RARs, specifically, loss of RAR- β expression, has been demonstrated in cervical cancer cell lines (15, 36–38). The present study revealed that the expression of all three RARs was decreased in CIN lesions. This finding is reminiscent of our findings that expression of RAR- β was lost in premalignant oral lesion and in head and neck cancers (24, 25).

Although not so directly related to retinoid activity as their receptors, the other proteins may play important roles in retinoid ability to prevent or delay malignant progression. TGF- β is a potent growth inhibitor for normal and initiated keratinocytes *in vitro* (39, 40). Loss of TGF- β expression was associated with hyperproliferation and a high risk for malignant conversion of

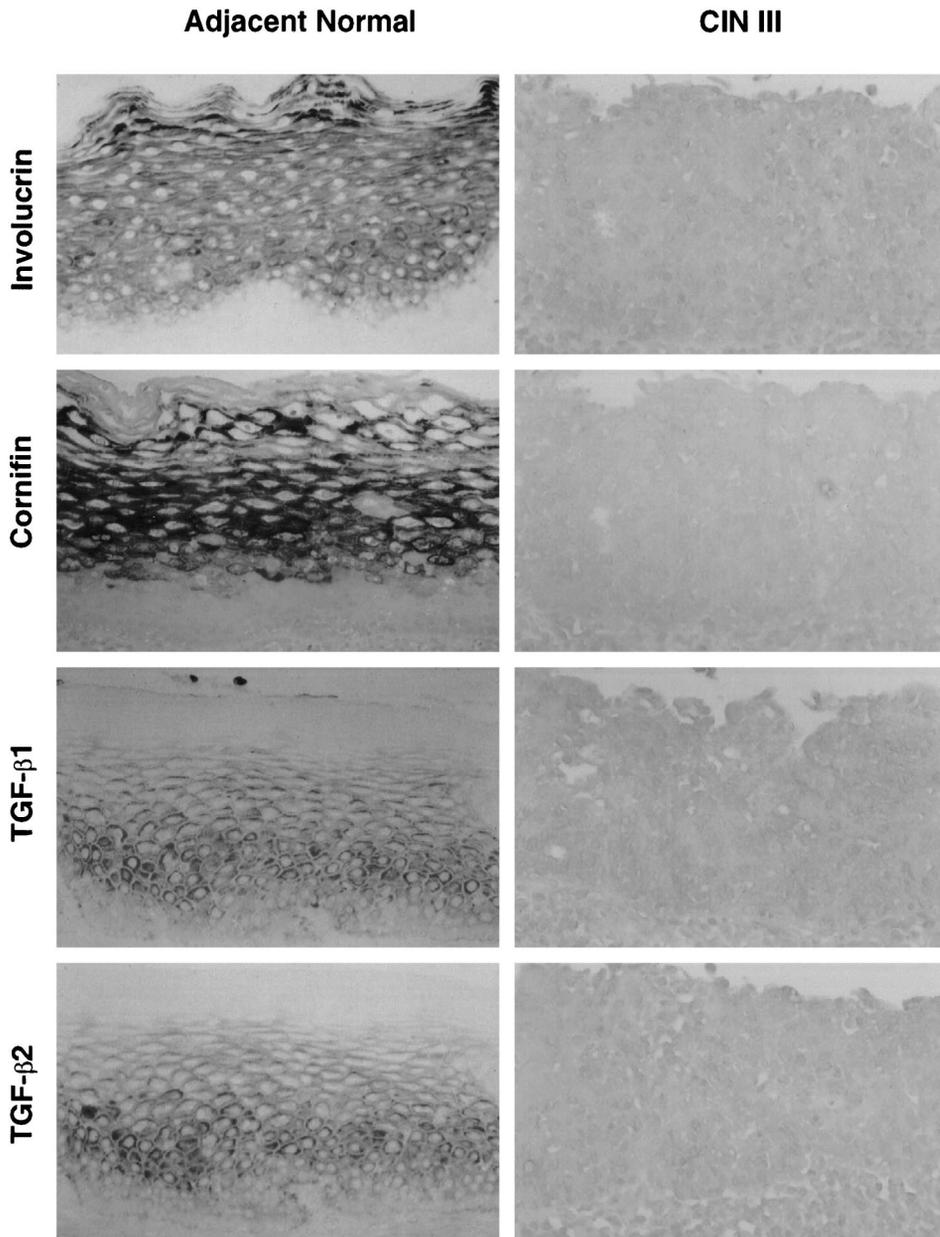


Fig. 2 Immunohistochemical detection of involucrin, cornifin, TGF- β 1, and TGF- β 2 in cervical specimen from core biopsy. Consecutive sections of a single paraffin block contained adjacent normal tissue, and CIN III were stained with antibodies against involucrin, cornifin, TGF- β 1, and TGF- β 2. Expression of these biomarkers was lost in CIN III.

skin and skin papilloma (41). In the present study, decreased expression of TGF- β 1 and TGF- β 2 was evident in all CIN lesions. This finding lends further support to a previous report, which demonstrated that loss of TGF- β 1 expression was an early event in the neoplastic transformation of cervical epithelia (42). Retinoids, however, induced secretion of latent TGF- β 1 and TGF- β 2 in normal and human papillomavirus type 16-immortalized human keratinocytes (11). Furthermore, infection with genital HPVs E6 and E7 is of critical importance for the cellular changes that precede cervical neoplasia as well as other genital cancers (43). Retinoids can decrease the HPV-immortalized rate of human keratinocytes and modulate the level or activity of E6 and E7 transforming proteins by either down-regulating HPV mRNA expression or inducing TGF- β secretion

in HPV-immortalized cells (11, 36, 44). It would be of interest to determine whether detection of TGF- β may serve as an intermediate end point biomarker in clinical trials of retinoid intervention in cervical carcinogenesis.

The relevance of involucrin and cornifin, which are protein components of cornified envelopes abundant in the upper spinous and granular layers of the epidermis, is their role as markers of squamous differentiation (32, 45, 46). We found that involucrin expression was reduced in CIN lesions compared with normal epithelia. This is in agreement with previous studies, in which it was reduced in both CIN and cervical cancer (45, 47, 48). Several studies have shown that RA can suppress squamous differentiation, including the level of involucrin mRNA and protein (31). Cornifin was recently cloned by Jet-

ten's group and its localization was very similar to involucrin in epidermis (32, 46). The present study is the first to report down-regulation of cornifin expression in CIN lesions. Like involucrin, cornifin levels are reduced by retinoids (46).

In conclusion, the present study demonstrated the expression patterns of nuclear retinoic acid receptors, involucrin, cornifin, TGF- β 1, and TGF- β 2 in CIN lesions and suggested that some of them might be useful biomarkers in clinical chemoprevention trials in cervical cancer development.

ACKNOWLEDGMENTS

We thank Susan Cweren for preparing the tissue sections and Drs. Wei Hu and Iouri Boiko for helping review the stained sections.

REFERENCES

- Morrow, C. P., and Cozen, W. Perspective on cervical cancer: why prevent? *J. Cell. Biochem. Suppl.*, *23*: 61–70, 1995.
- Morris, M., Tortolero-Luna, G., Malpica, A., Baker, V. V., Cook, E., Johnson, E., and Follen Mitchell, M. Cervical intraepithelial neoplasia and cervical cancer. *Obstet. Gynecol. Clin. N. Am.*, *23*: 347–410, 1996.
- Landis, S. H., Murray, T., Bolden, S., and Wingo, P. A. Cancer statistics 1999. *CA Cancer J. Clin.*, *49*: 8–31, 1999.
- Hong, W. K., and Sporn, M. B. Recent advances in chemoprevention of cancer. *Science (Washington DC)*, *278*: 1073–1077, 1997.
- Kelloff, G. J., Boone, C. W., Crowell, J. A., Nayfield, S. G., Hawk, E., Steele, V. E., Lubet, R. A., and Sigman, C. C. Strategies for phase II cancer chemoprevention trials: cervix, endometrium, and ovary. *J. Cell. Biochem. Suppl.*, *23*: 1–9, 1995.
- Mitchell, M. F., Hittelman, W. N., Lotan, R., Nishioka, K., Tortolero-Luna, G., Richards-Kortum, R., and Hong, W. K. Chemoprevention trials in the cervix: design, feasibility, and recruitment. *J. Cell. Biochem. Suppl.*, *23*: 104–112, 1995.
- Mitchell, M. F., Tortolero-Luna, G., Wright, T., Sarkar, A., Richards-Kortum, R., Hong, W. K., and Schottenfeld, D. Cervical human papillomavirus infection and intraepithelial neoplasia: a review. *J. Natl. Cancer Inst. Monogr.*, *21*: 17–25, 1996.
- Moon, R. C., and Mehta, R. G. Cancer chemoprevention by retinoids: animal models. *Methods Enzymol.*, *190*: 395–406, 1990.
- Lippman, S. M., Kavanagh, J. J., Paredes-Espinoza, M., Delgadillo-Madrueno, F., Paredes-Casillas, P., Hong, W. K., Holdener, E., and Krakoff, I. H. 13-*cis*-Retinoic acid plus interferon α -2a: highly active systemic therapy for squamous cell carcinoma of the cervix [see comments]. *J. Natl. Cancer Inst.*, *84*: 241–245, 1992.
- Lippman, S. M., Kavanagh, J. J., Paredes-Espinoza, M., Delgadillo-Madrueno, F., Paredes-Casillas, P., Hong, W. K., Massimini, G., Holdener, E. E., and Krakoff, I. H. 13-*cis*-Retinoic acid plus interferon- α 2a in locally advanced squamous cell carcinoma of the cervix. *J. Natl. Cancer Inst.*, *85*: 499–500, 1993.
- Batova, A., Danielpour, D., Pirisi, L., and Creek, K. E. Retinoic acid induces secretion of latent transforming growth factor β 1 and β 2 in normal and human papillomavirus type 16-immortalized human keratinocytes. *Cell Growth Differ.*, *3*: 763–772, 1992.
- Boone, C. W., and Kelloff, G. J. Intraepithelial neoplasia, surrogate endpoint biomarkers, and cancer chemoprevention. *J. Cell. Biochem. Suppl.*, *17F*: 37–48, 1993.
- 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*, pp. 597–658. New York: Raven Press, 1994.
- Meyskens, F. L., Jr., Surwit, E., Moon, T. E., Childers, J. M., Davis, J. R., Dorr, R. T., Johnson, C. S., and Alberts, D. S. Enhancement of regression of cervical intraepithelial neoplasia II (moderate dysplasia) with topically applied all-*trans*-retinoic acid: a randomized trial [see comments]. *J. Natl. Cancer Inst.*, *86*: 539–543, 1994.
- Lancillotti, F., Giandomenico, V., Affabris, E., Fiorucci, G., Romeo, G., and Rossi, G. B. Interferon α -2b and retinoic acid combined treatment affects proliferation and gene expression of human cervical carcinoma cells. *Cancer Res.*, *55*: 3158–3164, 1995.
- Eckert, R. L., Agarwal, C., Hembree, J. R., Choo, C. K., Sizemore, N., Andreatta-van Leyen, S., and Rorke, E. A. Human cervical cancer. Retinoids, interferon and human papillomavirus. *Adv. Exp. Med. Biol.*, *375*: 31–44, 1995.
- Lotan, R., Dawson, M. I., Zou, C. C., Jong, L., Lotan, D., and Zou, C. P. Enhanced efficacy of combinations of retinoic acid- and retinoid X receptor-selective retinoids and α -interferon in inhibition of cervical carcinoma cell proliferation. *Cancer Res.*, *55*: 232–236, 1995.
- Behbakht, K., DeGeest, K., Turyk, M. E., and Wilbanks, G. D. All-*trans*-retinoic acid inhibits the proliferation of cell lines derived from human cervical neoplasia. *Gynecol. Oncol.*, *61*: 31–39, 1996.
- 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*, pp. 319–350. New York: Raven Press, 1994.
- Chambon, P. A decade of molecular biology of retinoic acid receptors. *FASEB J.*, *10*: 940–954, 1996.
- Kelloff, G. J., Boone, C. W., Crowell, J. A., Steele, V. E., Lubet, R., and Doody, L. A. Surrogate endpoint biomarkers for phase II cancer chemoprevention trials. *J. Cell. Biochem. Suppl.*, *19*: 1–9, 1994.
- Ruffin, M. T. T., Ogaily, M. S., Johnston, C. M., Gregoire, L., Lancaster, W. D., and Brenner, D. E. Surrogate endpoint biomarkers for cervical cancer chemoprevention trials. *J. Cell. Biochem. Suppl.*, *23*: 113–124, 1995.
- Mitchell, M. F., Hittelman, W. K., Lotan, R., Nishioka, K., Tortolero-Luna, G., Richards-Kortum, R., Wharton, J. T., and Hong, W. K. Chemoprevention trials and surrogate end point biomarkers in the cervix. *Cancer (Phila.)*, *76*: 1956–1977, 1995.
- Xu, X. C., Ro, J. Y., Lee, J. S., Shin, D. M., Hong, W. K., and Lotan, R. Differential expression of nuclear retinoid receptors in normal, premalignant, and malignant head and neck tissues. *Cancer Res.*, *54*: 3580–3587, 1994.
- Lotan, R., Xu, X. C., Lippman, S. M., Ro, J. Y., Lee, J. S., Lee, J. J., and Hong, W. K. Suppression of retinoic acid receptor- β in premalignant oral lesions and its up-regulation by isotretinoin. *N. Engl. J. Med.*, *332*: 1405–1410, 1995.
- Lotan, R., Xu, X.-C., Zou, C., and Hong, W.-K. Retinoids and their receptors in modulation of differentiation, development, and prevention of head and neck cancer. *Ann. Oncol.*, *5* (Suppl. 9): S23–S28, 1994.
- Xu, X. C., Clifford, J. L., Hong, W. K., and Lotan, R. Detection of nuclear retinoic acid receptor mRNA in histological tissue sections using nonradioactive *in situ* hybridization histochemistry. *Diagn. Mol. Pathol.*, *3*: 122–131, 1994.
- Xu, X. C., Lee, J. S., Lippman, S. M., Ro, J. Y., Hong, W. K., and Lotan, R. Increased expression of cytokeratins CK8 and CK19 is associated with head and neck carcinogenesis. *Cancer Epidemiol. Biomark. Prev.*, *4*: 871–876, 1995.
- Park, T. K., Lee, J. P., Kim, S. N., Choi, S. M., Kudelka, A. P., and Kavanagh, J. J. Interferon- α 2a, 13-*cis*-retinoic acid and radiotherapy for locally advanced carcinoma of the cervix: a pilot study. *Eur. J. Gynaecol. Oncol.*, *19*: 353–358, 1998.
- Darwiche, N., Celli, G., Sly, L., Lancillotti, F., and De Luca, L. M. Retinoid status controls the appearance of reserve cells and keratin expression in mouse cervical epithelium. *Cancer Res.*, *53*: 2287–2299, 1993.
- Zou, C. P., Clifford, J. L., Xu, X. C., Sacks, P. G., Chambon, P., Hong, W. K., and Lotan, R. Modulation by retinoic acid (RA) of squamous cell differentiation, cellular RA-binding proteins, and nuclear RA receptors in human head and neck squamous cell carcinoma cell lines. *Cancer Res.*, *54*: 5479–5487, 1994.
- Jetten, A. M., De Luca, L. M., Nelson, K., Schroeder, W., Burlingame, S., and Fujimoto, W. Regulation of cornifin α expression in the vaginal and uterine epithelium by estrogen and retinoic acid. *Mol. Cell. Endocrinol.*, *123*: 7–15, 1996.

33. Sabichi, A. L., Hendricks, D. T., Bober, M. A., and Birrer, M. J. Retinoic acid receptor β expression and growth inhibition of gynecologic cancer cells by the synthetic retinoid *N*-(4-hydroxyphenyl) retinamide. *J. Natl. Cancer Inst.*, *90*: 597–605, 1998.
34. de The, H. Altered retinoic acid receptors. *FASEB J.*, *10*: 955–960, 1996.
35. Darwiche, N., Celli, G., and De Luca, L. M. Specificity of retinoid receptor gene expression in mouse cervical epithelia. *Endocrinology*, *134*: 2018–2025, 1994.
36. Bartsch, D., Boye, B., Baust, C., zur Hausen, H., and Schwarz, E. Retinoic acid-mediated repression of human papillomavirus 18 transcription and different ligand regulation of the retinoic acid receptor β gene in non-tumorigenic and tumorigenic HeLa hybrid cells. *EMBO J.*, *11*: 2283–2291, 1992.
37. Celli, G., Darwiche, N., and De Luca, L. M. Estrogen induces retinoid receptor expression in mouse cervical epithelia. *Exp. Cell Res.*, *226*: 273–282, 1996.
38. Geisen, C., Denk, C., Gremm, B., Baust, C., Karger, A., Bollag, W., and Schwarz, E. High-level expression of the retinoic acid receptor β gene in normal cells of the uterine cervix is regulated by the retinoic acid receptor α and is abnormally down-regulated in cervical carcinoma cells. *Cancer Res.*, *57*: 1460–1467, 1997.
39. Sporn, M. B., Roberts, A. B., Wakefield, L. M., Glick, A. B., and Danielpour, D. Transforming growth factor- β and suppression of carcinogenesis. *Princess Takamatsu Symp.*, *20*: 259–266, 1989.
40. Glick, A. B., Sporn, M. B., and Yuspa, S. H. Altered regulation of TGF- β 1 and TGF- α in primary keratinocytes and papillomas expressing v-Ha-ras. *Mol. Carcinog.*, *4*: 210–219, 1991.
41. Glick, A. B., Kulkarni, A. B., Tennenbaum, T., Hennings, H., Flanders, K. C., O'Reilly, M., Sporn, M. B., Karlsson, S., and Yuspa, S. H. Loss of expression of transforming growth factor β in skin and skin tumors is associated with hyperproliferation and a high risk for malignant conversion. *Proc. Natl. Acad. Sci. USA*, *90*: 6076–6080, 1993.
42. Comerci, J. T., Jr., Runowicz, C. D., Flanders, K. C., De Victoria, C., Fields, A. L., Kadish, A. S., and Goldberg, G. L. Altered expression of transforming growth factor- β 1 in cervical neoplasia as an early biomarker in carcinogenesis of the uterine cervix. *Cancer (Phila.)*, *77*: 1107–1114, 1996.
43. Lorincz, A. T., Reid, R., Jenson, A. B., Greenberg, M. D., Lancaster, W., and Kurman, R. J. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. *Obstet. Gynecol.*, *79*: 328–337, 1992.
44. Woodworth, C. D., Notario, V., and DiPaolo, J. A. Transforming growth factors β 1 and 2 transcriptionally regulate human papillomavirus (HPV) type 16 early gene expression in HPV-immortalized human genital epithelial cells. *J. Virol.*, *64*: 4767–4775, 1990.
45. Sassoon, A. F., Said, J. W., Nash, G., Shintaku, I. P., and Banks-Schlegel, S. Involucrin in intraepithelial and invasive squamous cell carcinomas of the cervix: an immunohistochemical study. *Hum. Pathol.*, *16*: 467–470, 1985.
46. Marvin, K. W., George, M. D., Fujimoto, W., Saunders, N. A., Bernacki, S. H., and Jetten, A. M. Cornifin, a cross-linked envelope precursor in keratinocytes that is down-regulated by retinoids. *Proc. Natl. Acad. Sci. USA*, *89*: 11026–11030, 1992.
47. Auersperg, N., Kruk, P. A., MacLaren, I. A., Watt, F. M., and Myrdal, S. E. Heterogeneous expression of keratin, involucrin, and extracellular matrix among subpopulations of a poorly differentiated human cervical carcinoma: possible relationships to patterns of invasion. *Cancer Res.*, *49*: 3007–3014, 1989.
48. Nair, S. A., Nair, M. B., Jayaprakash, P. G., Rajalekshmy, T. N., Nair, M. K., and Pillai, M. R. Involucrin and tumor progression in the uterine cervix. *Pathobiology*, *64*: 333–338, 1996.