The Effect of Cellular Retinoic Acid Binding Protein-I Expression on the CYP26-Mediated Catabolism of All-*Trans* Retinoic Acid and Cell Proliferation in Head and Neck Squamous Cell Carcinoma

Jun Yeon Won, Eui-Cheol Nam, Seung Joo Yoo, Hyun Ja Kwon, Soo Jong Um, Hye Sook Han, Sun Hee Kim, Youngro Byun, and Sang Yoon Kim

The aim of this study was to confirm if catabolism of all-*trans* retinoic acid (RA) is enhanced by type I cellular retinoic acid binding protein (CRABP-I) expression and to investigate the effect of this enhanced catabolism on cell proliferation of the head and neck squamous cell carcinoma (HNSCC) cell line, AMC-HN-7. We also analyzed the effects of CRABP-I on RA-induced retinoic acid receptor (RAR) activity. The expression of the CRABP-I in stably transfected AMC-HN-7 cell lines (HN7-BPIa and HN7-BPIb) resulted in a lower sensitivity to administered RA compared with that of controls in a clonogenic assay. HN7-BPIs cells showed an increased amount of polar metabolites of RA in thin-layer chromatography. The transcriptional activity of the reporter plasmid RARE(DR5)-tk-CAT after the treatment of RA was lesser in HN7-BPIs than in controls. These results suggest that the increased CYP26-mediated catabolism of RA by CRABP-I transfection might decrease the amount of RA that is accessible to the nuclear receptors and make HNSCC cells resistant to RA.
© 2004 Elsevier Inc. All rights reserved.

RETINOIC ACID, a type of vitamin A derivative, has been known to suppress carcinogenesis in various epithelial tissues[1] and to inhibit cell proliferation and differentiation of squamous cell carcinomas.[2,3] The biologic activities of retinoic acid are believed to be mediated by the nuclear retinoic acid receptors (RARs and RXRs), all of which are ligand-inducible transcriptional factors that regulate the expression of target genes.[4]

Each of the 2 subtypes of retinoid receptors, RARs and RXRs, includes 3 isotypes designated α, β, and γ. RARs can form heterodimers with RXRs, and RXRs can also form homodimers. Such dimers can bind to RA-response elements (RARE) in the regulatory regions of certain targets. Activation of transcription by RAR-RXR and RXR-RXR dimers is usually mediated via DR5 (RARE) and DR1 (RXRE), respectively.[4,5]

A class of intracellular proteins that bind retinoic acids with a high affinity comprises 2 homologous proteins, cellular retinoic acid binding proteins I and II (CRABP-I and CRABP-II). Although the 2 proteins share a high degree of homology, they differ in spatial and temporal expression patterns, suggesting that each type of protein may have distinct functions. It has been shown that overexpression of CRABP-II enhances cellular response to retinoic acid in breast cancer cells,[6] but overexpression of CRABP-I decreases the effect of retinoic acid in F9 teratocarcinoma cells.[7] Fiorella and Napoli[8] reported that holo-CRABP is a substrate for retinoic acid catabolism in rat testes microsomes.

Microsomal P450s (P450) play an important role in retinoic acid metabolism and maintenance of vitamin A homeostasis. All-*trans* retinoic acid (RA), which is known as an active metabolite of vitamin A, can be catabolized by human retinoic acid acid 4-hydroxylase (CYP26).[9] Kim et al[10] reported that P450 could be induced by RA in some AMC-HN cell lines in which RA was rapidly catabolized into polar metabolites. Among that series of HNSCC cell lines, AMC-HN-7 showed a sensitive response to RA and delayed catabolism of added RA.[10,11] AMC-HN-7 cells expressed CYP26 before and after the treatment of RA, but did not express CRABP-I.[11]

We believe that the deficiency of CRABP-I in AMC-HN-7 cells contributes to the delayed catabolism and the potent effect of RA. We therefore performed stable transfection of the human CRABP-I gene in AMC-HN-7 cell lines and investigated any consequent changes of the cellular response to RA. The aim of this study was to confirm if CRABP-I expression enhances catabolism of RA and to investigate the effect of this enhanced catabolism on cell proliferation of HNSCCs.

MATERIALS AND METHODS

Cell Maintenance

We established a HNSCC cell line, AMC-HN-7, at our laboratory from patients with HNSCC.[12] The cells were maintained in Eagle's minimum essential medium (MEM) supplemented with 1% nonessential amino acid, 2 mmol/L L-glutamine, and 10% fetal bovine serum (FBS) (Gibco, Life Technologies, Grand Island, NY). After gene transfection, G418 (Gibco) was added to media at a concentration of 400 μg/mL. All cultures were incubated at 37°C in a humidified atmosphere with 5% CO_2.

hCRABP-I Gene Transfection

We established 2 stably transfected AMC-HN-7-hCRABP-I (HN7-BPIa and b) cell lines expressing hCRABP-I by using a pcDNA3.1(-) vector. The hCRABPI/pSVL vectors (human cDNA of CRABP-I

From the Department of Otolaryngology, University of Kangwon College of Medicine, Chunchon; Department of Otolaryngology, Asan Medical Center, College of Medicine, University of Ulsan, Seoul; Department of Bioscience and Biotechnology/Institute of Bioscience, Sejong University, Seoul; Department of Clinical Pathology, Samsung Medical Center, College of Medicine, Sungkyunkwan University, Seoul; and the Department of Materials Science and Engineering, Kwangju Institute of Science and Technology, Kwangju, Korea.
Submitted July 22, 2003; accepted December 10, 2003.
Supported by Grant No. HMP-98-G-2-050-B from the 1998 Highly Advanced National Projects on the Development of Biomedical Engineering and Technology, Ministry of Health and Welfare, R.O.K and Grant No. 02-PJ1-PG10-20599-0003 from the Ministry of Health and Welfare, R.O.K.
Address reprint requests to Sang Yoon Kim, MD, Department of Otolaryngology, Asan Medical Center, University of Ulsan College of Medicine, 388-1, Pungnap-dong, Songpa-gu, Seoul 138-736, Korea.
© 2004 Elsevier Inc. All rights reserved.
0026-0495/04/5308-0033$30.00/0
doi:10.1016/j.metabol.2003.12.015

cloned at 5′ XbaI - BamHI 3′ in pSVL (Promega, Madison, WI)) were kindly provided by Dr Anders Åström (Astrazeneca R&D, Lund, Sweden). After the separation of insert cDNA, the cloning was performed between 5′ XbaI - BamHI 3′ in a pCDNA3.1(-) vector (Invitrogen, Carlsbard, CA). The vectors were transfected to AMC-HN-7 cells with a LipofectAMINE PLUS Reagent (Gibco-BRL).

Reverse Transcriptase-Polymerase Chain Reaction Analysis

The cells treated with 1 μmol/L RA (Sigma Chemical, St Louis, MO) for 24 hours. Total RNA was isolated by the Trizol reagent (Gibco) from the cells that were transfected with pCDNA3.1(-) vector alone (HN7-vector) as controls and from the established cell lines, HN7-BPIa and HN7-BPIb. Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using the following primers for the CRABP-I and CYP26 genes. The sense primer of the CRABP-I gene was 5′-CGG CAC CTG GAA GAT GCG CA-3′ and the antisense primer was 5′-CCA CGT CAT CGG CGC CAA ACTTG-3′. Forty cycles of the PCR were run as follows: 94°C, 45 seconds; 65°C, 45 seconds; 72°C, 90 seconds; and the final extension at 72°C for 10 minutes. The sense primer of the CYP26 gene was 5′-TCC TCG CAC AAG CAG CGA AAG AAG GTG ATT-3′ and the antisense primer was 5′-ATG TGG GTA GAG TCC TAG GTA AGT-3′. Thirty-five cycles of the PCR were run as follows: 94°C, 30 seconds; 60°C, 1 minute; 72°C, 1 minute; and the final extension at 72°C for 10 minutes. Primers for human β-actin were used as a control.

Western Blot Analysis

Protein was extracted from the cells that were transfected with pCDNA3.1(-) vector alone (HN7-vector) as controls and from the established cell lines, HN7-BPIa and HN7-BPIb. The samples were loaded onto 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA). The membrane was blocked with 5% blocking reagent for 1 hour at room temperature. After 2 washes with 0.1% phosphate-buffered saline (PBS)-T (PBS with 0.1% Tween 20 [Sigma]), the blot was incubated with 1:1,000 monoclonal (mouse) anti-CRABP-I antibody (IgG2b; Affinity Bioreagents, Golden, CO) for 1 hour. The membrane was washed twice with 0.1% PBS-T and incubated with 1:3,000 dilution of horseradish peroxidase-labeled antibody (Amersham, Buckinghamshire, UK) for 1 hour. The membrane was developed using an elecrochemiluminescence (ECL) western kit (Amersham) and placed in a film cassette for exposure to scientific imaging film. The film was developed and the protein bands were analyzed.

Thin-Layer Chromatography

Cells were characterized for enzymatic conversion of 33 nmol/L all-trans-[11,12-^{3}H (N)]-RA ([^{3}H]-RA) to polar metabolites as previously described.[13] Cells were treated with RA (1 μmol/L) for 16 hours in the dark and rinsed 3 times with PBS. Cells were then treated with 0.05% trypsin, 0.02% EDTA for 8 minutes and rinsed 4 times with PBS by centrifugation for 5 minutes at 5,000 $\times$ g. Cells were next resuspended in a homogenate buffer (0.5 mol/L sucrose, 10 mmol/L Tris-Cl [pH 7.4], 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 0.1 μg/mL leupeptin, and 0.04 U/mL aprotinin) and homogenized in a tissue grinder. The homogenate was diluted with an equal volume of 10 mmol/L Tris-Cl (pH 7.4) and 1 mmol/L EDTA, laid over a 0.5 vol homogenation buffer, and then centrifuged at 9,000 $\times$ g for 10 minutes at 4°C. The supernatant was recentrifuged at 9,000 $\times$ g to remove cell debris, nuclei, and mitochondria. The final supernatant was centrifuged at 100,000 $\times$ g for 45 minutes at 4°C. The pellet was resuspended in a storage buffer (0.25 mol/L sucrose, 10 mmol/L Tris-Cl [pH 7.4], 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 0.1 μg/mL leupeptin, and 0.04 U/mL aprotinin) and stored at −20°C.

Approximately 1 mg of microsomal proteins was incubated with 33 nmol/L [^{3}H]-RA (Du Pont-New England Nuclear, Boston, MA) in 600 μL PBS at 37°C for 1 hour in the dark. Reactions were terminated by addition of 600 μL chloroform and methanol (2:1), and the reacted solution was centrifuged for 5 minutes at 5,000 $\times$ g. The organic phase containing retinoids was collected and dried under a nitrogen atmosphere in the dark. Dried extracts were resuspended in ethanol (25 to 100 μL) and analyzed by thin-layer chromatography (TLC).

Dissolved sampled (25 μL in ethanol) and standard RA were applied to the TLC plates (LK6D silica gel; Whatman, Hillsboro, OR) in the dark. TLC plates were developed for 90 minutes in a glass tank preequilibrated for 1 hour with 150 mL developing solvent (hexane: ether:acetic acid, 90:60:1.5 vol/vol/vol). The glass tank contained 1 sheet of solvent-saturated Whatman No. 1 paper. TLC plates were air-dried for 5 minutes, and then the standard RA were marked on the plates. TLC plates were then sprayed with [^{3}H]enhancer (Du Pont), followed by air-drying for 2 hours, and exposed to the X-OMAT film (Kodak, Rochester, NY) for 12 hours at −80°C to localize the retinoids. Radiolabeled RA metabolites on the TLC plates were scraped and their radioactivities were measured.

Clonogenic Assay

One hundred cells were plated onto 6-well plates with a 2-mL G418 MEM media. The cells were allowed to attach for 24 hours before replacement of the G418 MEM media with 0.1 μmol/L RA, 1 μmol/L RA, 10 μmol/L RA, and with dimethyl sulfoxide (DMSO) as a control. RA was melted in 10 μL DMSO at a concentration of 10 mmol/L and serially diluted to the desired final concentrations in growth media. Control cultures received the same amount of DMSO as RA-treated cultures.

After 3 days, the culture media were replaced with the MEM media and the cells were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 3 days. Colonies were stained with 4% crystal violet and the numbers of colonies per well were counted. Statistical significance was determined using the Wilcoxon rank sum test. We considered values of $P \leq .05$ statistically significant.

Chloramphenicol Acetyltransferase Assay

The cells (2 $\times$ 10^5) were seeded on 60-mm dishes and cultivated for 24 hours. The cells were cotransfected with 1.5 μg DNA, which was made up of 1 μg reporter plasmid RARE(DR5)-tk-chloramphenicol acetyltransferase (CAT) and 0.5 μg β-galactosidase plasmid. After an overnight incubation in the presence of DNA precipitates, the cells were washed once with PBS and 1 μmol/L concentration of RA was added. RA was melted in 10 μL DMSO at a concentration of 10 mmol/L and made 1 μmol/L of solution with MEM media. After 24 hours of incubation, the cells were suspended in a lysis buffer (0.25 mol/L Tris-HCl, pH 7.5) and were disrupted by sonication.

The lysates were centrifuged and the supernatants were used for a CAT assay. CAT activity was measured with a CAT enzyme-linked immunosorbent assay (ELISA) kit (Boehringer-Mannheim, Germany) as indicated by the manufacturer. Protein concentrations were determined using a protein assay kit (Bio-Rad). The CAT activity of individual samples was normalized for transfection efficiency by the corresponding β-galactosidase activity. β-galactosidase activity was measured spectrophotometrically at 420 nm and was used as an internal standard to normalize the CAT activity. Statistical significance was determined using the Wilcoxon rank sum test. We considered values of $P \leq .05$ statistically significant.

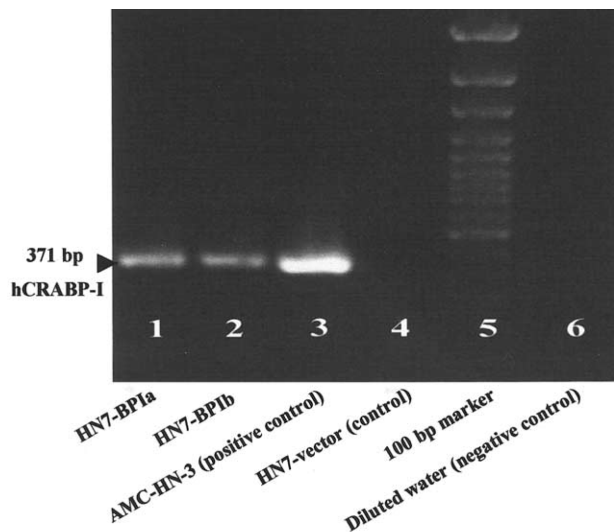

Fig 1. The expression of CRABP-I in RT-PCR. HN7-BPIa (lane 1) and HN7-BPIb (lane 2) cell lines expressed CRABP-I mRNA. But the HN7-vector cell lines (lane 4) as the control did not express CRABP-I mRNA. Strong expression of CRABP-I in AMC-HN-3 cells was detected (lane 3). Diluted water was used as the negative control (lane 6).

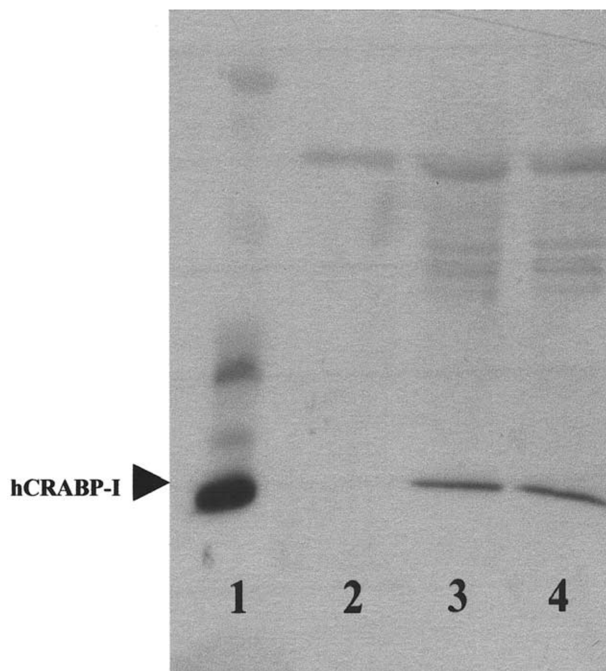

Fig 2. Western blot analysis of CRABP-I protein. No signal was detected in the HN7-vector cell lines (lane 2), whereas CRABP-I was detected in the HN7-BPIa (lane 3) and HN7-BPIb (lane 4) cell lines. Strong expression of CRABP-I in AMC-HN-3 cells was detected (lane 1).

RESULTS

Identification of Transfected AMC-HN-7 Cell Lines

The HN7-vector cell lines did not express CRABP-I in RT-PCR (Fig 1) and Western blot analysis (Fig 2). But 2 stably transfected AMC-HN-7-hCRABP-I (HN7-BPIa and b) cell lines expressed CRABP-I in RT-PCR (Fig 1) and Western blot analysis (Fig 2). We confirmed stable transfection of CRABP-I in HN7-BPIa and HN7-BPIb cells. The HN7-vector cell lines and HN7-BPIs (HN7-BPIa and HN7-BPIb) cell lines expressed CYP26 without RA treatment. And RA markedly increased the expression of CYP26 in all the cell lines (Fig 3). These results indicate that CYP26 was induced by RA in AMC-HN-7 cell lines regardless of transfection.

RA Metabolism and Cell Proliferation

RA-treated HN7-BPIs cell lines showed an increased amount of polar metabolites of RA in TLC. But, RA-treated HN7-vector cells did not show an increased amount of polar metabolites of RA in TLC. Strong bands of polar metabolites in RA-treated AMC-HN-6 cells (positive control)[10] were detected (Fig 4). This result means that the transfected CRABP-I increased the CYP26-mediated catabolism of RA.

The fractions of colony formation of HN7-BPIa, HN7-BPIb, and HN7-vector in the clonogenic assay with the treatment of RA 1μmol/L for 6 days were 81%, 89%, and 66%, respectively ($P < .05$ between HN7-vector cells and HN7-BPIa cells, $P < .01$ between HN7-vector cells and HN7-BPIb cells) (Fig 5). The expressions of the CRABP-I in HN7-BPIs cell lines resulted in a lower sensitivity to the administered RA relative to that of the control cell line (HN7-vector).

The increasing amount of the activity of the reporter plasmid RARE(DR5)-tk-CAT after the treatment of RA was lesser in

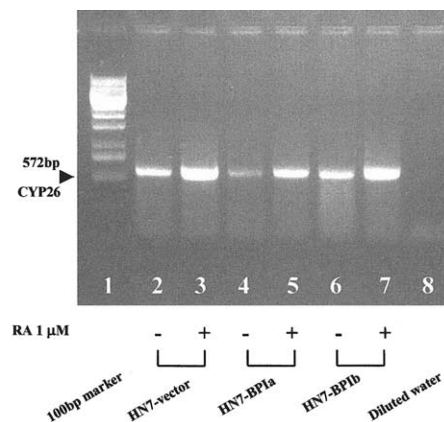

Fig 3. The expression of CYP26 in the RT-PCR. The HN7-vector cell lines and the HN7-BPIs cell lines expressed CYP26 mRNA before RA treatment. After the cells were treated with 1 μmol/L RA for 1 day, the expression of CYP26 was stronger than that of the untreated cells in all the cell lines. Diluted water was used as the negative control (lane 8).

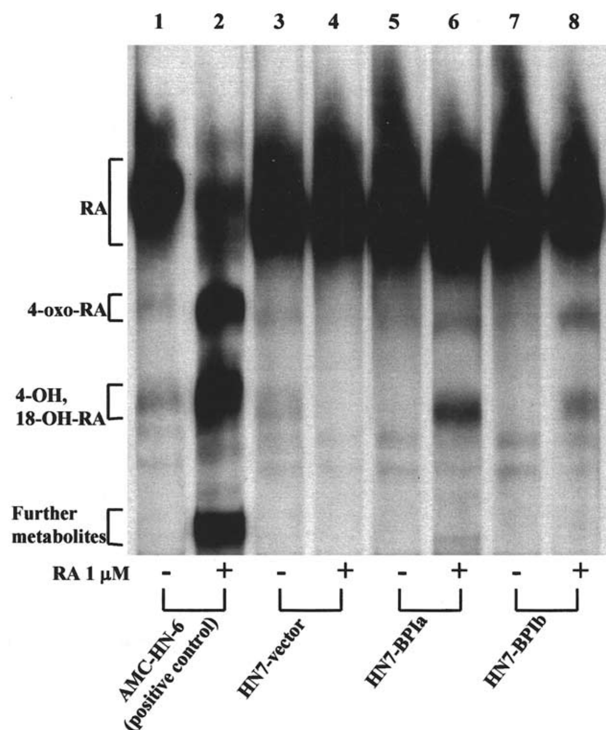

Fig 4. A TLC separation profile of RA metabolites. The upper strong band on the TLC plate was RA and the several bands below RA were polar metabolites, which included 4-OH-RA, 18-OH-RA, and 4-oxo-RA. Polar metabolites were detected in RA-treated HN7-BPIs (HN7-BPIa and HN7-BPIb cell lines) cells (lanes 6 and 8), but not in HN7-vector cells (lane 4). An increment of polar metabolites in RA-treated AMC-HN-6 cells (positive control) was noticed (lane 2).

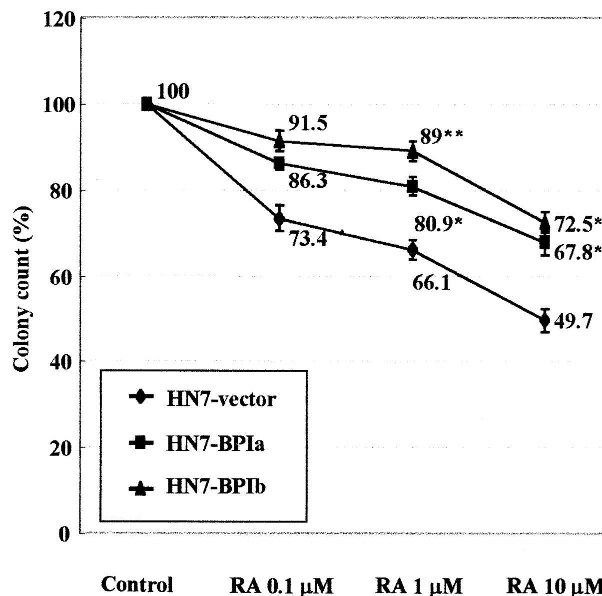

Fig 5. The results of colony-forming efficacy. HN7-vector cells formed a fewer number of colonies than did HN7-BPIs (HN7-BPIa and HN7-BPIb cell lines) cells after RA treatment (*$P < .05$, **$P < .01$). Data are shown as the mean ± SD (n = 9).

HN7-BPIs than in the HN7-vector. The ratio of the reporter gene expression in HN7-BPIa, HN7-BPIb, and HN7-vector with RA 1μmol/L for 24 hours increased to 107.0% ± 14.8%, 99.7% ± 36.5%, and 182.1% ± 38.0%, respectively ($P = .05$ between HN7-vector cells and HN7-BPIa cells, $P = .05$ between HN7-vector cells and HN7-BPIb cells) (Fig 6). Cells overexpressing CRABP-I exhibited a lower transcriptional activation of RARE(DR5) driven reporter after treatment of RA than cells transfected with vector alone. This indicates that the overexpressed CRABP-I decreased the response of the cells to retinoid signaling.

These results indicate that the overexpressed CRABP-I in the HNSCC cell line increases the catabolism of RA, decreases the amount of RA that is accessible to the nuclear receptors, and results in a lower sensitivity to RA.

DISCUSSION

RA can induce human RA 4-hydroxylase (CYP 26) in some cells, which is known to be highly specific for RA.[9] The major metabolites of RA are the 4- and 18-OH-RA. The 4-OH-RA formed from RA may be further converted to 4-oxo-RA.[13] The CYP26 activity may be one essential factor for the RA sensitivity, but in cells showing induction of CYP26, the RA sensitivity is inversely related to the rate of RA catabolism.[14] The data presented in Fig 4 demonstrate that the overexpression of CRABP-I of transfected AMC-HN-7 cell lines shows an increased amount of polar metabolites of RA in TLC. Hence, catabolism of RA is considered to be enhanced by CRABP-I.

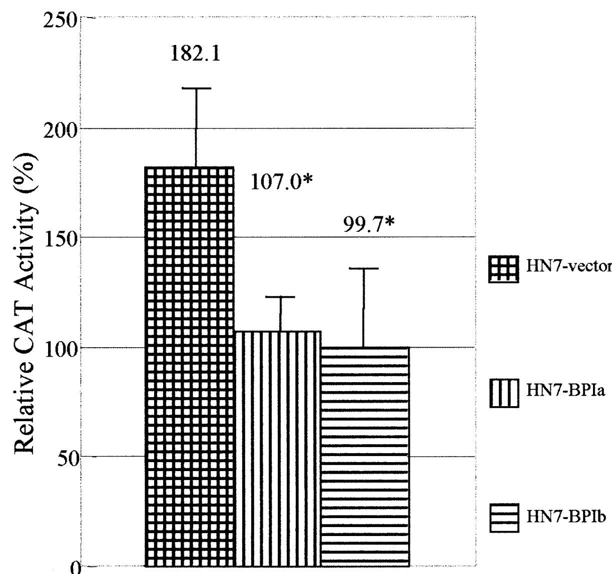

Fig 6. Different transcriptional response of CRABP-I expressing cells to RA. Cells were cotransfected with RARE(DR5)-tk-CAT reporter and β-galactosidase control genes. After treated with RA, the transcriptional activity of the reporter gene was analyzed by a CAT ELISA as described. The transcriptional activity of the reporter plasmid RARE(DR5)-tk-CAT after the treatment of RA was lesser in HN7-BPIs than in controls (*$P = .05$). Data are shown as the mean ± SD (n = 3).

The CRABPs (CRABP-I and CRABP-II) have been known to regulate intracellular RA concentration and the expression pattern of CRABPs is various in HNSCCs.[2] Boylan and Gudas[7] reported that the level of CRABP-I determined the rate of RA catabolism. Giannini et al[2] reported that the RA-resistant SCC cell line, CCL-17, did not express CRABP-I. However, CRABP-I may not be directly involved in the retinoid receptor-mediated RA-signaling pathway.[15] CRABP-II, which shows a large increase after introduction of RA, is generally thought to be a protein that mediates the intracellular regulatory activities of RA.[16] Dong et al[17] reported that movement of RA from CRABP-II to the receptor is facilitated by a mechanism that involves direct interactions between CRABP-II and RAR. In 1992, Busch et al[18] reported that topical application of RA decreased CRABP-I and increased CRABP-II expression in keratinocytes.

In vitro biochemical studies have shown that CRABP-I can participate in catabolic reactions for RA, suggesting a role of this protein in the cytosolic events responsible for retinoid metabolism.[8,19,20] Boylan and Gudas[7] reported that overexpression of the CRABP-I protein in stably transfected F9 stem cell lines resulted in a lower sensitivity to a given external concentration of RA. In this study, stably transfected AMC-HN-7 cell lines with CRABP-I showed a decreased sensitivity to RA in clonogenic assay. These results mean that the sensitivity of cells to RA was influenced by overexpression of CRABP-I, which caused the increased catabolism of RA. But Braakhuis et al[21] reported that the extent of metabolism was proportional to the degree of growth suppression and suggested that the ability of HNSCC cells to metabolize RA is related not to resistance, but rather to a growth-inhibitory effect. However, Kim et al[14] reported that RA sensitivity is inversely related to the rate of RA catabolism. In addition, van der Leede et al[22] reported that RA was a more active metabolite, and the mechanism that activates the rapid catabolism might prevent the cells from continuous exposure to RA. Therefore, we suggest that RA, rather than its metabolites, might be the major effector of cells.

The increasing amount of activity of the reporter plasmid RARE(DR5)-tk-CAT after the treatment of RA was lesser in stably transected AMC-HN-7 cell lines with CRABP-I than in untransfected AMC-HN-7 cell lines. This indicates that the function of CRABP-I in the HNSCC cell is to mediate the catabolism of RA and to regulate the amount of RA that is accessible to the nuclear receptors. In conclusion, the increased CYP26-mediated catabolism of RA by CRABP-I transfection might decrease the amount of RA that is accessible to the nuclear receptors and makes HNSCC cells resistant to RA.

ACKNOWLEDGMENT

We thank Dr Anders Åström (Astrazeneca R&D, Lund, Sweden) for providing the hCRABPI/pSVL vectors.

REFERENCES

1. Moon RC, Mehta RG: Retinoid: Inhibition of experimental carcinogenesis, in Dawson MI, Okamura WH (eds): Chemistry and Biology of Synthetic Retinoids. Boca Raton, FL, CRC, 1990, pp 501-518

2. Giannini F, Maestro R, Vukosavljevic T, et al: All-trans, 13-cis and 9-cis retinoic acids induce a fully reversible growth inhibition in HNSCC cell lines: Implications for in vivo retinoic acid use. Int J Cancer 70:194-200, 1997

3. Shalinsky DR, Bischoff ED, Gregory ML, et al: Retinoid-induced suppression of squamous cell differentiation in human oral squamous cell carcinoma xenografts (line 1483) in athymic nude mice. Cancer Res 55:3183-3191, 1995

4. Mangelsdorf DJ, Umesono K, Evans RM: Cellular retinoid binding proteins, in Sporn MB, Roberts AB, Goonman DS (eds): The Retinoids; Biology, Chemistry, and Medicine (ed 2). New York, NY, Raven, 1994, pp 319-349

5. Chambon P: A decade of molecular biology of retinoic acid receptors. FASEB J 10:940-954, 1996

6. Jing Y, Waxman R, Mira-y-Lopez R: The cellular retinoic acid binding protein II is a positive regulator of retinoic acid signaling in breast cancer cells. Cancer Res 57:1668-1672, 1997

7. Boylan JF, Gudas LJ: Overexpression of the cellular retinoic acid binding protein-I (CRABP-I) results in a reduction in differentiation-specific gene expression in F9 teratocarcinoma cells. J Cell Biol 112: 965-979, 1991

8. Fiorella PD, Napoli JL: Expression of cellular retinoic acid binding protein in Escherichia coli: Characterization and evidence that holo-CRABP is a substrate in retinoic acid metabolism. J Biol Chem 266:16572-16579, 1991

9. Sonneveld E, van den Brink CE, van der Leede BM, et al: Human retinoic acid (RA) 4-hydroxylase (CYP26) is highly specific for all-trans-RA and can be induced through RA receptors in human breast and colon carcinoma cells. Cell Growth Differ 9:629-637, 1998

10. Kim SY, Han IS, Yu HK, et al: The induction of P450-mediated oxidation of all-*trans* retinoic acid by retinoids in head and neck squamous cell carcinoma cell lines. Metabolism 47:955-958, 1998

11. Yoo SJ, Kwon HJ, Nam SY, et al: Effect and catabolism of all-trans retinoic acid in head and neck squamous cell carcinoma. Korean J Otolaryngol 42:1541-1546, 1999

12. Kim SY, Chu KC, Lee HR, et al: Establishment and characterization of nine new head and neck cancer cell lines. Acta Otolaryngol 117:775-784, 1997

13. Han IS, Choi J-H: Highly specific cytochrome P450-like enzymes for all-trans-retinoic acid in T47D human breast cancer cells. J Clin Endocrinol Metab 81:2069-2075, 1996

14. Kim SY, Yoo SJ, Kwon HJ, et al: Retinoic acid 4-hydroxlase-mediated catabolism of all-trans retinoid acid and the cell proliferation in head and neck squamous cell carcinoma. Metabolism 51:477-481, 2002

15. Venepally P, Reddy LG, Sani BP: Analysis of the effects of CRABP I expression on the RA-induced transcription mediated by retinoid receptors. Biochemistry 35:9974-9982, 1996

16. Vo HP, Crowe DL: Transcriptional regulation of retinoic acid responsive genes by cellular retinoic acid binding protein-II modulates RA mediated tumor cell proliferation and invasion. Anticancer Res 18:217-224, 1998

17. Dong D, Ruuska SE, Levinthal DJ, et al: Distinct roles for cellular retinoic acid-binding proteins I and II in regulating signaling by retinoic acid. J Biol Chem 274:23695-23698, 1999

18. Busch C, Siegenthaler G, Vahlquist A, et al: Expression of cellular retinoid-binding proteins during normal and abnormal epidermal differentiation. J Invest Dermatol 99:795-802, 1992

19. Ong DE: Cellular transport and metabolism of vitamin A: Roles of the cellular retinoid-binding proteins. Nutr Rev 52:S24-S31, 1994

20. Boylan JF, Gudas LJ: The level of CRABP-1 expression influences the amounts and types of all-trans-retinoic acid metabolites in F9 teratocarcinoma stem cells. J Biol Chem 267:21486-21491, 1992

21. Braakhuis BJM, Klassen I, van der Leede BM, et al: Retinoid metabolism and all–*trans* retinoic acid-induced growth inhibition in head and neck squamous cell carcinoma cell lines. Br J Cancer 76:189-197, 1997

22. van der Leede BM, van den Brink CE, Pijnappel WW, et al: Autoinduction of retinoic acid metabolism to polar derivatives with decreased biological activity in retinoic acid-sensitive, but not in retinoic acid-resistant human breast cancer cells. J Biol Chem 272:17921-17928, 1997