Analysis of Retinoic Acid Receptor β Expression in Normal and Malignant Laryngeal Mucosa by a Sensitive and Routine Applicable Reverse Transcription-Polymerase Chain Reaction Enzyme-linked Immunosorbent Assay Method[1]

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ABSTRACT

Retinoic acid receptor β (RAR-β) seems to be a useful intermediate marker in trials of retinoids, and the aim of this work was to describe a fast, sensitive, and routine applicable method to measure *RAR*-β expression in tumor biopsies. We developed a new technique combining reverse transcription-PCR with a colorimetric ELISA detection of amplification products. The principle of this nonradioactive method is based on digoxigenin labeling of PCR products during amplification. Amplified DNA is hybridized with a biotinylated capture probe. The generated hybrid is immobilized on a streptavidin-coated microtiter plate, and detection is performed with the use of an antidigoxigenin peroxidase conjugate. We applied this method to quantify the expression of *RAR*-β and an internal control (*β2 microglobulin*) in laryngeal tumors. We found a detection threshold at 50 pg of PCR products, which represents a 100-fold improvement when compared to the detection limit of ethidium bromide detection. The method was reproducible (intra- and interassay reproducibilities at 7 and 5%, respectively). We used this technique for determining *RAR*-β expression in 20 patients with laryngeal carcinoma and in 20 patients without cancer. The data show that the value of the RAR-β:β2 microglobulin ratio is decreased in tumoral *versus* nontumoral specimens ($P = 0.0012$), which is consistent with previously published results.

Received 2/27/97; revised 5/10/97; accepted 6/16/97.

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[1] Supported in part by La Ligue Nationale de Lutte contre le Cancer, Comité du Var.

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INTRODUCTION

Retinoids, including vitamin A and its analogues, are involved in epithelial cell differentiation *in vitro* and *in vivo* (1–3). This property was used in chemoprevention for head and neck squamous cell carcinoma (4, 5), and several clinical studies have shown that retinoids can reverse premalignant lesions of the oral cavity (6–8) and the uterine cervix (9) or prevent the appearance of second primary tumors (10, 11). This retinoid effect is mediated by RARs[3] and retinoid X receptors, which belong to the thyroid/steroid hormone receptor family. Each of the receptor types has at least three subtypes, α, β, and γ (1, 12).

RAR-β, which acts as a transcriptional factor through binding to a RA-responsive element, is inducible on RA action (13). Numerous studies have shown that *RAR*-β expression is decreased in various tumor-derived cell lines *in vitro* (14–18). Lotan and coworkers have demonstrated that a decrease in *RAR*-β expression can also be observed *in vivo* in malignant and premalignant lesions of head and neck cancer (19). Furthermore, these authors have shown that 13-*cis*-RA treatment of patients with premalignant oral lesions induces an increase in the proportion of specimens expressing RAR-β associated with clinical response (8).

Different methods have been described to determine *RAR*-β expression, including Northern blot, *in situ* hybridization, immunoprecipitation of ligand-receptor complexes with specific antipeptide, and RT-PCR (18–21). Northern blot analysis and immunoprecipitation generally require a large amount of tissues (100 and 150 mg, respectively). In addition, Northern blotting does not seem to be sufficiently sensitive for the detection of RAR-β transcripts in tumoral specimens (20). *In situ* hybridization was successfully used for RAR-β quantification (19) but is cumbersome to apply for routine use. Finally, some studies have described the use of RT-PCR analysis for determination of *RAR*-β expression (18, 20). In these cases, PCR products were separated by gel electrophoresis, blotted onto a membrane, and hybridized with a *RAR*-β probe. These last steps are time-consuming and are not easily compatible with routine applicability.

Because *RAR*-β cellular expression seems to be an indicator of malignancy and a useful intermediate marker in trials of retinoids, the aim of our work was to develop a sensitive

[3] The abbreviations used are: RAR, retinoic acid receptor; RT, reverse transcription; RA, retinoic acid; nt, nucleotide.

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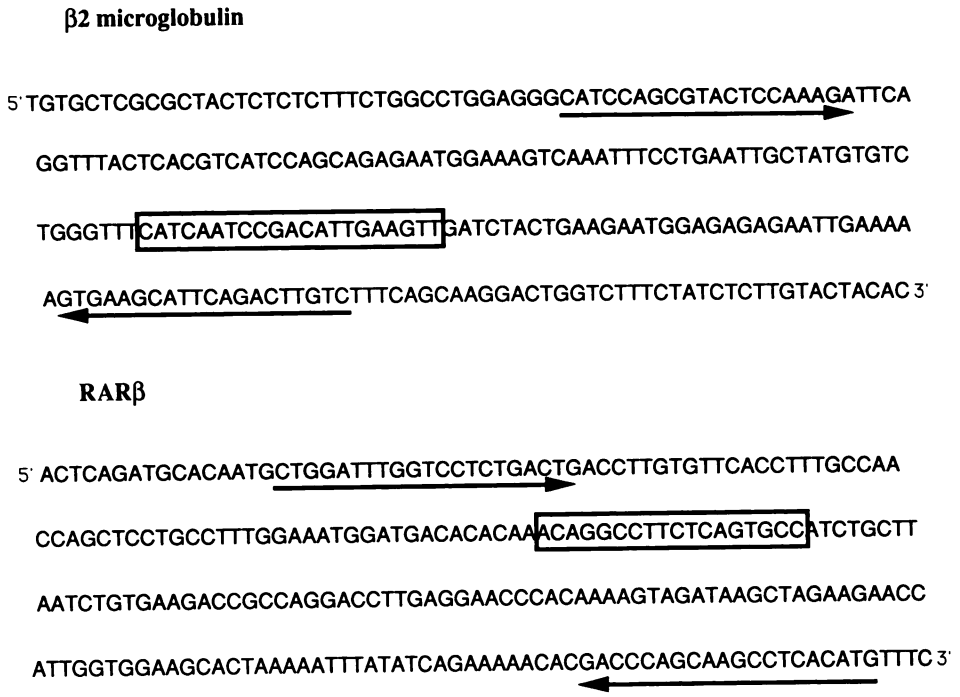

Fig. 1 Primers and capture probes used for PCR ELISA. *Arrows*, primers used for PCR amplification. *Boxed sequences*, capture probes used for ELISA detection. These probes are 5′ biotinylated.

technique for *RAR*-β expression quantification that could be routinely applicable for the exploration of small tissue samples. We report a semiquantitative method including RT-PCR associated with ELISA detection for quantification of *RAR*-β expression in tumoral biopsies. This technique was clinically applied for the analysis of 20 tumoral or 20 nontumoral larynx tissue samples.

MATERIALS AND METHODS

Tissue Samples. Biopsy specimens were obtained from 20 patients with laryngeal carcinoma and from 20 patients operated on for benign congenital abnormalities of the larynx with no risk factors (nonsmokers, no alcohol consumption). The mean age of the study population was 56.2 years, ranging from 30–71 years. The tumor-node-metastasis classification was used for the histopathological grading in the tumor group. Patients were distributed as follows: 3 (15%) stage I; 7 (35%) stage II; 5 (25%) stage III; and 5 (25%) stage IV. All biopsies were performed at the time of direct laryngoscopy under general anesthesia. A fraction of the biopsy specimens were placed in cryoconservation tubes and immersed in liquid nitrogen for further study of the RAR-β gene expression. The remaining part of the specimen was used for histological examination. Histological studies confirmed the malignancy in the tumor group and the presence of normal mucosa in the control group. Biopsy specimens weighed between 10 and 30 mg.

Plasmids and Primers. A plasmid containing the human *RAR*-β cDNA was used as a positive control for PCR. The oligonucleotides (Eurobio) used for β*2 microglobulin* amplification were: μ1, 5′-CATCCAGCGTACTCCAAAGA-3′ (nt 97–116); and μ2, 5′-GACAAGTCTGAATGCTCCAC-3′ (nt 242–261). The oligonucleotides used for *RAR*-β amplification were: β1, 5′-CTGGATTTGGTCCTCTGACT-3′ (nt 1199–1217); and β2, 5′-CATGTGAGGCTTGCTGGGTC-3′ (nt 1400–1419; Fig. 1). Both primer pairs span an intron to distinguish the PCR products generated from cDNA and genomic DNA. Two capture probes (Eurobio) corresponding respectively to the inner part of β*2 microglobulin* PCR product 5′-CATCAATCCGACATTGAAGTT-3′ (nt 188–217) and to the inner part of *RAR*-β PCR product 5′-ACAGGCCTTCTCAGTGCC-3′ (nt 1276–1293) were used for ELISA detection (Fig. 1). Both probes were 5′ biotinylated and purified by high-performance liquid chromatography.

RNA Extraction and RT. Total RNA was extracted using a method derived from Chomczynski (RNA NOW kit; Biogentex). RNA quality was checked by gel electrophoresis, and quantification was performed by densitometric analysis. RNA (3 μg) was reverse-transcribed in the presence of random hexamers in a 60-μl final volume. Briefly, RNA was incubated at 65°C for 5 min in 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 1.25 mM each of four deoxyribonucleotide triphosphates, and 5 μM random hexamers (Life Technologies, Inc., Cergy-Pontoise, France) and immediately cooled on ice. After the addition of 600 units of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) and 250 units of human placenta RNase inhibitor (Amersham, Les Ulis, France), the reaction mix was incubated at 42°C for 30 min and at 94°C for 5 min.

PCR Conditions. RNA equivalent (250 ng) was submitted to PCR amplification in a 100-μl final volume including 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each of the deoxyribonucleotide triphosphates, 5 units of Taq polymerase (Boehringer Mannheim, Meylan, France), and 250

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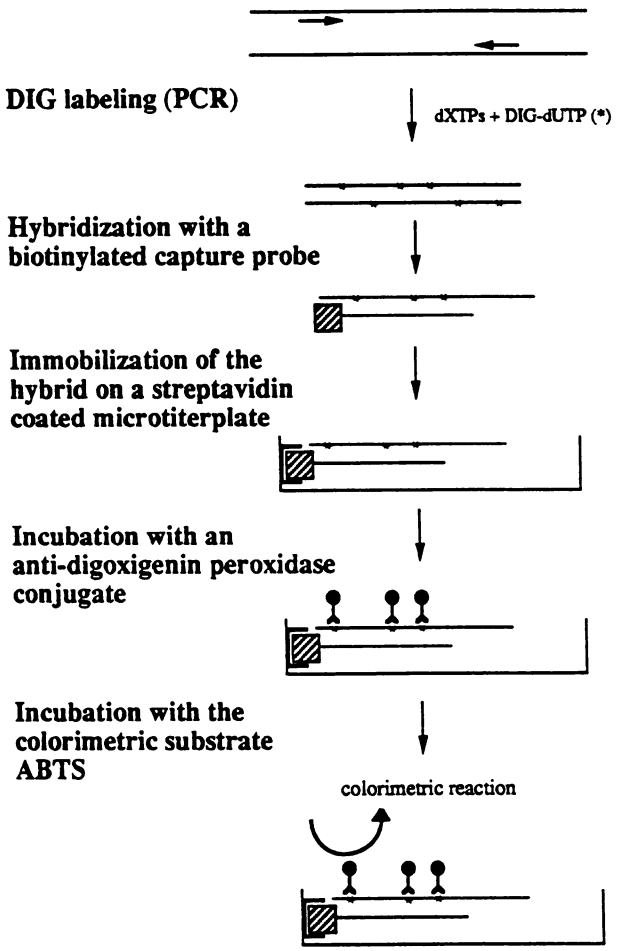

Fig. 2 Principles of PCR ELISA.

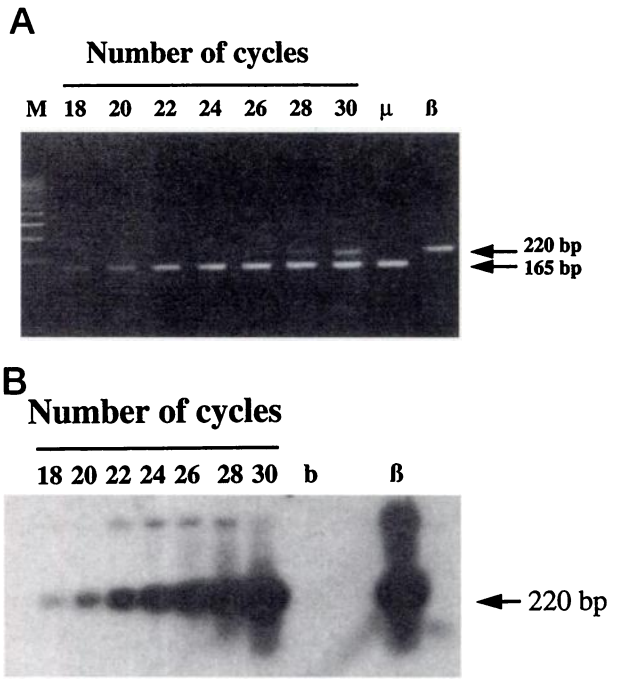

Fig. 3 Amplification kinetics of *RAR*-β and β*2 microglobulin*. Total RNA extracted from a head and neck tumoral specimen was reverse-transcribed using random hexamers. Aliquots of cDNA (250 ng of RNA equivalent) were submitted to PCR amplification for the indicated number of cycles in the presence of both primer pairs β1/β2 and μ1/μ2. *A*, 20 μl of PCR products were analyzed on a 2% agarose gel. *M*, molecular weight DNA marker; β, amplification using the *RAR*-β plasmid as template; μ, amplification using the 165-bp β*2 microglobulin* DNA fragment as template. *B*, 10 μl of the same PCR products were analyzed by Southern blotting with the *RAR*-β plasmid as a probe.

nM of both primer pairs μ1/μ2 and β1/β2. The amplification reaction using a thermal cycler (Perkin-Elmer Corp., St Quentin-en-Yvelines, France) consisted of an initial 5-min incubation at 94°C, followed by *n* (see "Results") amplification cycles (94°C for 1 min, 58°C for 1 min, and 72°C for 30 s).

PCR ELISA. The principle of this technique is presented in Fig. 2. After digoxigenin labeling of PCR products during amplification, amplified DNA is hybridized with a biotinylated capture probe corresponding to the inner part of PCR product. The generated hybrid is immobilized on a streptavidin-coated microtiter plate, and detection is performed by the use of an antidigoxigenin peroxidase conjugate.

Digoxigenin labeling of PCR products was performed by using the PCR ELISA DIG labeling kit (Boehringer Mannheim). The labeling reaction was carried out during coamplification of *RAR*-β and β*2 microglobulin* for an optimal number of cycles (see "Results") in the presence of digoxigenin-labeled dUTP using the conditions described above and following the manufacturer's recommendations. Detection of the PCR products was performed by using the PCR ELISA DIG detection kit (Boehringer Mannheim). Briefly, 80 (RAR-β detection) or 1 μl (β2 microglobulin detection) of PCR products was denatured by the addition of 40 μl of an alkaline solution. The reaction mixture was then completed to 500 μl with the hybridization solution containing the *RAR*-β or β*2 microglobulin* capture probe at 7.5 nM. Two hundred μl of this reaction mix were transferred to a streptavidin-coated well of a microtiter plate and incubated at 45°C for 1 h under shaking. After the addition of an antidigoxigenin peroxidase conjugate, the microtiter plate was incubated at 37°C for 30 min under shaking. The detection was performed by the addition of the colorimetric substrate ABTS and incubation at 37°C for 1 h. The absorbance was read at 405 nm in an ELISA reader.

The detection threshold of the method was determined by analyzing serial dilutions of PCR products previously quantified by comparison with DNA molecular weight markers after gel electrophoresis. Interassay reproducibility of the method was measured by analyzing *RAR*-β expression in a tumoral sample (cDNA) in three independent experiments. Intra-assay reproducibility was determined by analyzing *RAR*-β expression of the same tumoral sample six times within the same experiment.

Southern Blotting. Ten-μl aliquots of the amplification products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide. After transfer to a Hybond N membrane (Amersham), hybridization was performed with a

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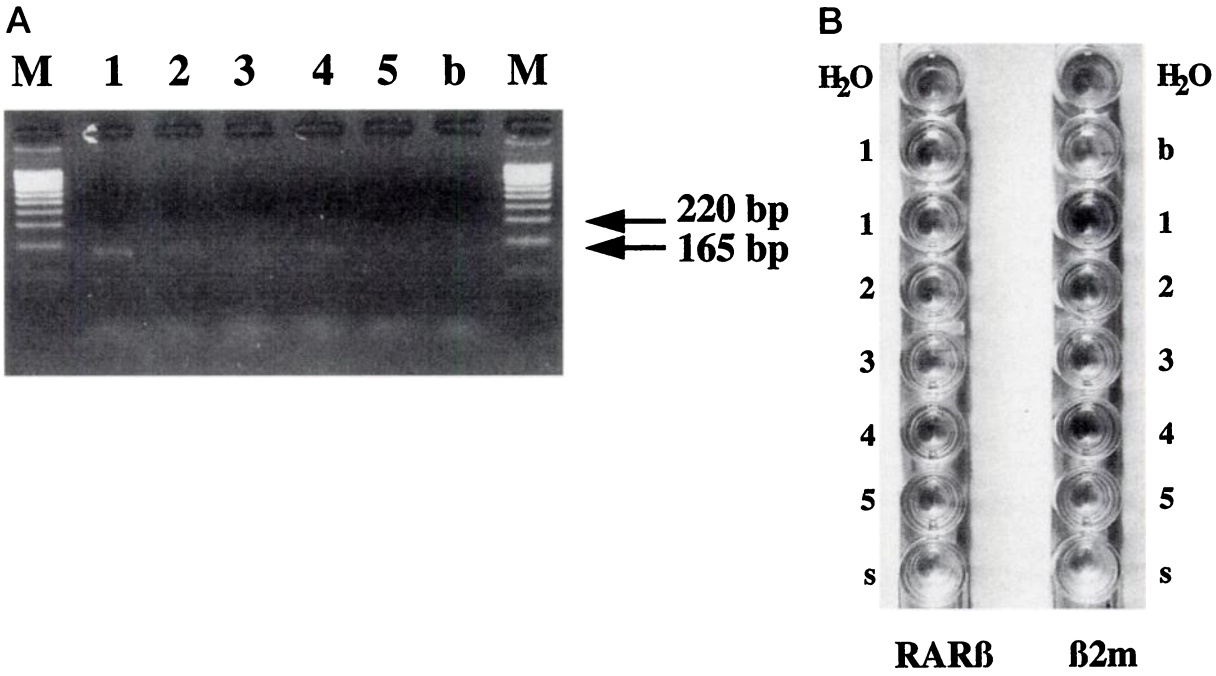

Fig. 4 Analysis of tumoral samples. *A*, analysis by gel electrophoresis of 10 μl of PCR products obtained after 21 cycles of amplification. *Lanes 1–5*, tumoral specimens from different patients; *M*, molecular weight DNA marker; *b*, control without DNA. *B*, ELISA detection of the same PCR products with the indicated capture probe. A total of 80 (RAR-β detection) or 1 (β2 microglobulin detection) μl of amplified DNA was hybridized with *RAR*-β or β2 *microglobulin* capture probes and submitted to ELISA detection. *Samples 1–5*, tumoral specimens; *b*, control without DNA; *s*, ABTS substrate alone. Sample 1 was voluntarily analyzed in duplicate.

probe consisting of ^{32}P-labeled *RAR*-β plasmid according to standard procedures (22).

RESULTS

Semiquantitative RT-PCR Assay. To determine the expression level of the RAR-β gene in a semiquantitative manner, we developed a RT-PCR assay using the β2 microglobulin as an internal standard. For this purpose, we first analyzed the amplification kinetics of both genes in a coamplification reaction. Fig. 3*A* shows the analysis by gel electrophoresis of an aliquot of PCR products obtained after a variable number of cycles. Although the 165-bp β2 microglobulin-amplified fragment can be visualized after 18 cycles, the 220-bp DNA fragment corresponding to RAR-β is undetectable up to 26 cycles of amplification. This observation led us to analyze RAR-β PCR products after Southern blotting and hybridization with a ^{32}P-labeled RAR-β probe (Fig. 3*B*). Under these conditions, densitometric analysis of β2 microglobulin and RAR-β signals showed that: (*a*) both genes were amplified with comparable kinetics; and (*b*) coamplification must be carried out at 21 cycles to avoid the β2 microglobulin plateau (data not shown).

Use of PCR ELISA for Sensitive Detection of PCR Products. Due to the weak expression of RAR-β in tumoral samples, RAR-β PCR products obtained after 21 cycles of amplification are generally undetectable after electrophoresis on ethidium bromide-stained gels (Figs. 3*A* and 4*A*). Fig. 4*A* shows the analysis by gel electrophoresis of PCR products obtained after coamplification of RAR-β and β2 microglobulin for 21 cycles in the presence of digoxigenin-labeled dUTP. The 220-bp RAR-β-amplified DNA cannot be visualized in these different tumor specimens, and only a very faint band corresponding to the 165-bp β2 microglobulin fragment can be detected in some samples. Fig. 4*B* shows the ELISA colorimetric reaction of the same amplified DNA fragments. An absorbance of each well was easily measured, and the detection threshold could be estimated at 50 pg of PCR products. The intra- and interassay reproducibilities were 7 (n = 6) and 5% (n = 3), respectively.

Determination of RAR-β Expression Level in Tumoral *Versus* Nontumoral Tissues. RAR-β expression was analyzed in 20 tumoral and 20 nontumoral larynx tissue samples by the RT-PCR ELISA method. RAR-β and β2 microglobulin expression seemed to be measurable in all samples. The results are expressed as the ratio of the optical densities obtained for RAR-β and β2 microglobulin. Fig. 5 shows the distribution of the values of the ratio for tumoral or nontumoral specimens. The median value of the ratio was 450 (mean, 538.1 ± 79.1) and 926 (mean, 947.3 ± 102.8) in tumoral and nontumoral samples, respectively (P = 0.0012).

DISCUSSION

There are experimental data on cell lines (14–18) and observations in clinical samples from tumors of the head and neck (19) and lung (15, 23) showing that the tissular expression

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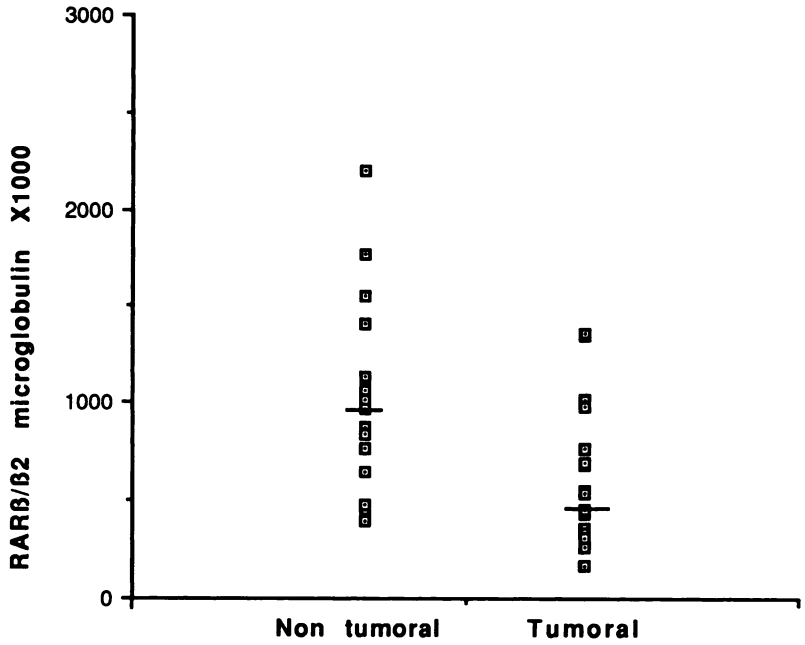

Fig. 5 Distribution of the RAR-β:β2 microglobulin ratio in tumoral and nontumoral samples. The median value is represented by a *horizontal line* on the graph.

of RAR-β is decreased with malignant development. Lotan *et al.* (8) found a selective loss of RAR-β mRNA expression in premalignant oral lesions. Interestingly, Houle *et al.* (24) showed that lung cancer cell lines transfected with the RAR-β gene were less tumorigenic in nude mice than the untransfected cells and showed reduced growth rate and increased latency. On the other hand, clinical data showed that treatment with retinoids can restore the level of RAR-β tissular expression (8). Together, these data point out that the measurement of RAR-β expression can be useful as both a molecular reflection of malignant development and a marker used to follow the treatment by retinoids. This underlines the need for an analytical method that could be easily applicable on a large scale in the clinical context. The methods currently proposed for measuring RAR-β expression are not really compatible with this purpose because of a lack of sensitivity, as with Northern blot (20) and immunoprecipitation (21), or a relative heaviness, as with *in situ* hybridization (19).

RT-PCR ELISA applications have been described in virology; for instance, the quantitative detection of hepatitis C virus (25). RT-PCR ELISA is based on the principle of a nonradioactive labeling of amplified products during the amplification step. Furthermore, the use of a capture probe allows the introduction of an additional level of specificity.

Our present approach is the first one applying RT-PCR ELISA for both the parameter of interest and the internal control (β2 microglobulin). A first advantage of the RT-PCR ELISA method adapted to the determination of RAR-β expression in malignant and normal oral mucosa is its increased sensitivity when compared to the classical detection of RT-PCR products with ethidium bromide staining (Fig 4, *A* and *B*). We found a detection threshold with the RT-PCR ELISA method at 50 pg of PCR product, which represents a 100-fold improvement when compared to the detection limit around 5 ng of the ethidium bromide detection via gel electrophoresis (26). The method is reproducible, because both intra- and interassay reproducibilities gave acceptable coefficient of variation values at 7 and 5%, respectively. Another advantage of the present method is its routine applicability using microtitration plates. One of the main features of the assay is the possible automation of PCR product quantification in microtiter plates, based on the use of a capture probe.

The clinical use of the method was tested by measuring RAR-β expression in 40 specimens from different mucosa samples obtained from patients with laryngeal carcinoma (n = 20) and from control patients without cancerous disease (n = 20). A significant decrease in the distribution of RAR-β expression was observed in tumoral tissue when compared to normal mucosa (Fig. 5). Although the main objective of the present study was not to perform a large-scale exploration of RAR-β expression in tumors, this observation nevertheless corroborates the results of the investigation by Xu *et al.* (19) showing a decreased expression of RAR-β in malignant head and neck tissue in comparison to normal oral mucosa.

Retinoids are currently being used to treat human malignancies and premalignant conditions (27). As emphasized by Hong *et al.* (5), one of the most important issues in aerodigestive cancer is the development of chemoprevention trials with retinoids, especially Phase III trials that evaluate cancer incidence. To reach this objective, a large number of samples are needed, with the use of biological markers among which RAR-β has a determinant role. RT-PCR ELISA could be a suitable method to measure RAR-β expression in this type of analysis.

ACKNOWLEDGMENTS

We are indebted to G. Carle for critical reading of the manuscript and to S. Michel for the gift of the RAR-β plasmid and helpful advice concerning RAR-β analysis by RT-PCR.

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REFERENCES

1. De Lucca, L. M. Retinoids and their receptors in differentiation, embryogenesis, and neoplasia. FASEB J., *5:* 2924–2933, 1991.

2. Gudas, L. J. Retinoids, retinoid-responsive genes, cell differentiation, and cancer. Cell Growth Differ., *3:* 655–662, 1992.

3. Lotan, R., Xu, X. C., Zou, C. P., and Hong, W. K. Retinoids and their receptors in modulation of differentiation, development, and prevention of head and neck cancer. Ann. Oncol., *5:* S23–S28, 1994.

4. Lippman, S. M., Benner, S. E., and Hong, W. K. Cancer chemoprevention. J. Clin. Oncol., *12:* 851–873, 1994.

5. 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.

6. Hong, W. K., Endicott, J., Itri, L. M., Doos, W., Batsakis, J. G., Bell, R., Fofonoff, S., Byers, R., Atkinson, E. N., Vaughan, C., Toth, B. B., Kramer, A., Dimery, I. W., Skipper, P., and Strong, S. 13-*cis*-Retinoic acid in the treatment of oral leukoplakia. N. Engl. J. Med., *315:* 1501–1505, 1986.

7. Lippman, S. M., Batsakis, J. G., Toth, B. B., Weber, R. S., Lee, J. J., Martin, J. W., Hays, G. L., Goepfert, H., and Hong, W. K. Comparison of low-dose isotretinoin with beta carotene to prevent oral carcinogenesis. N. Engl. J. Med., *328:* 15–20, 1993.

8. 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.

9. Meyskens, F. L., Surwit, E., Jr., 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. J. Natl. Cancer Inst., *86:* 539–543, 1994.

10. Hong, W. K., Lippman, S. M., Itri, M. M., Karp, D. D., Lee, J. S., Byers, R. M., Schantz, S. P., Krammer, A. M., Lotan, R., Peters, L. J., Dimery, I. W., Brown, D. W., and Goepfert, H. Prevention of second primary tumors with isotretinoid in squamous cell carcinoma of the head and neck. N. Engl. J. Med., *323:* 795–801, 1990.

11. Benner, S. E., Pajak, T. F., Lippman, S. M., Earley, C., and Hong, W. K. Prevention of second primary tumors with isotretinoid in patients with squamous cell carcinoma of the head and neck: long-term follow-up. J. Natl. Cancer Inst., *86:* 140–141 1994.

12. 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, pp. 319–349. New York: Raven Press, Ltd., 1994.

13. De Thé, H., Vivanco-Ruiz, M., Tiollais, P., Stunnenberg, H., and Dejean, A. Identification of a retinoic acid-responsive element in the retinoic acid receptor β gene. Nature (Lond.), *343:* 177–180, 1990.

14. Hu, L., Crowe, D. L., Rheinwald, J. G., Chambon, P., and Gudas, L. J. Abnormal expression of retinoic acid receptors and keratin K19 by human oral and epidermal squamous cell carcinoma cell lines. Cancer Res., *51:* 3972–3981, 1991.

15. Gebert, J. F., Moghal, N., Frangioni, J. V., Sugarbaker, D. J., and Neel, B. G. High frequency of the retinoic acid receptor β abnormalities in human lung cancer. Oncogene, *6:* 1859–1868, 1991.

16. Swisshelm, K., Ryan, K., Lee, X., Tsou, H. C., Peacocke, M., and Sager, R. Down-regulation of retinoic acid receptor β in mammary carcinoma cell lines and its up-regulation in senescing normal mammary epithelial cells. Cell Growth Differ., *5:* 133–141, 1994.

17. Zou, C., 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.

18. Hoffman, A. D., Engelstein, D., Bogenrieder, T., Papandreou, C. N., Steckelman, E., Dave, A., Motzer, R. J., Dmitrovski, E., Albino, A. P., and Nanus, D. M. Expression of retinoic acid receptor β in human renal cell carcinomas correlates with sensitivity to the antiproliferative effects of 13-*cis*-retinoic acid. Clin. Cancer Res., *2:* 1077–1082, 1996.

19. 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.

20. Geradts, J., Chen, J. Y., Russel, E. K., Yankaskas, J. R., Nieves, L., and Minna, J. D. Human lung cancer cell lines exhibit resistance to retinoic acid treatment. Cell Growth Differ., *4:* 799–809, 1993.

21. Titcomb, M. W., Gottardis, M. M., Pike, J. W., and Allegretto, E. A. Sensitive and specific detection of retinoid receptor subtype proteins in cultured cell and tumor extracts. Mol. Endocrinol., *8:* 870–877, 1994.

22. Sambrook, J., Fritsch, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual, 2nd edition. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.

23. Lotan, R., Sozzi, G., Ro, J., Lee, J., Pastorino, U., Pilotti, S., Kurie, J., Hong, W., and Xu, X. Selective suppression of retinoic acid receptor β (RARβ) expression in squamous metaplasia and in non-small cell lung cancers compared to normal bronchial epithelioma. Proc. Am. Soc. Clin. Oncol., *14:* 165, 1995.

24. Houle, B., Rochette-Egly, C., and Bradley, W. E. C. Tumor-suppressive effect of the retinoic acid receptor β in human epidermoid lung cancer cells. Proc. Natl. Acad. Sci. USA, *90:* 985–989, 1993.

25. Clossais Besnard, N., and Andre, P. M. Automated quantitative determination of hepatitis C virus viremia by reverse transcription-PCR. J. Clin. Microbiol., *32:* 1887–1893, 1994.

26. Katz, E. D., and Dong, M. W. Rapid analysis and purification of polymerase chain reaction products by high-performance liquid chromatography. Biotechniques, *8:* 546–555, 1990.

27. Claxton, D. F., Plunkett, W., Andreeff, M., and Deisseroth, A. B. Retinoids and cancer therapy. J. Natl. Cancer Inst., *84:* 1306–1307, 1992.

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Clin Cancer Res 1997;3:2137-2142.

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