Cyclooxygenase-2 Overexpression Is a Marker of Poor Prognosis in Stage I Non-Small Cell Lung Cancer[1]

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ABSTRACT

Cyclooxygenase-2 (COX-2), the enzyme that converts arachidonic acid to prostaglandins, is overexpressed in a variety of different tumors, including those of the colon, pancreas, lung, and head and neck. We used *in situ* hybridization with a digoxgenin-labeled COX-2 antisense riboprobe to assess the presence of strong or intermediate *versus* weak or absent COX-2 expression in specimens from 160 patients with stage I non-small cell lung cancer (NSCLC). Of these, 3 specimens had strong expression, 69 had intermediate expression of COX-2, 24 had weak expression, and 64 had no detectable COX-2. The strength of COX-2 expression was associated with a worse overall survival rate ($P = 0.001$) and a worse disease-free survival rate ($P = 0.022$). The median survival times for the strong, intermediate or weak, and null COX-2 expressors were 1.04, 5.50, and 8.54 years, respectively. Interestingly, all three specimens with strong COX-2 expression came from patients who died within 18 months. Retinoic acid receptor β (RAR-β) is a nuclear retinoid receptor whose expression is frequently lost in aerodigestive tract carcinogenesis. We previously demonstrated that expression of RAR-β in stage I NSCLC indicates a poor prognosis. Retinoids have been shown to prevent induction of COX-2 by mitogens and tumor promoters. Expression of COX-2 correlated with RAR-β expression ($P = 0.053$), but not with k-*ras* mutational status, vascular endothelial growth factor, basic fibroblast growth factor, interleukin 8 levels, or other markers of angiogenesis, invasion, and metastases. Thus, like RAR-β positivity, COX-2 overexpression appears to portend a shorter survival among patients with early stage non-small cell lung cancer. Future studies of RAR-β and COX-2 regulation in NSCLC should further the development of prevention and therapy interventions with retinoids and/or COX-2 antagonists in this patient population.

INTRODUCTION

Lung cancer is by far the leading cause of cancer-related death. Predictions are that 164,100 new cases of lung cancer and 156,900 deaths from lung cancer will occur in the United States in 2000 (1). The 5-year survival rate for lung cancer has improved from 9% in 1963 to a plateau at 14% in 1994, but because of the 86% lethality of this diagnosis, there is a strong need for new and better control of lung cancer (2).

Assessment of molecular prognostic factors in patients with NSCLC[3] (3), in the hope that the identification of molecular risk factors will lead to individually tailored strategies, is one area of intense research. Particular focus has been to identify these factors in stage I disease. The standard of therapy for patients with stage I disease is complete surgical resection, and outcome has previously been correlated with the presence or absence of certain prognostic factors (3–6). Thus, the goal of this avenue of investigation is to identify the patients with stage I NSCLC who are both at highest risk and most likely to benefit from adjuvant or chemopreventive therapies and offer such therapy to those patients. Toward this end, several major molecular markers have been evaluated in association with established histological, clinical, and radiographic prognostic parameters of NSCLC (7–11). Slebos *et al.* (7) found that the presence of k-*ras* mutations indicates a poor prognosis in cases of surgically resectable NSCLC. Furthermore, in a study of 164 patients with NSCLC who underwent resection, our group showed that the loss of blood-group antigen A also indicates a poor prognosis (8).

Conversely, our data on the prognostic significance of RAR-β were surprising. Our evaluation of 156 patients with stage I NSCLC yielded a surprising finding, namely, that strong expression of RAR-β was found in 41 (26%) patients and that these patients had a significantly ($P = 0.045$) worse overall survival and a trend toward a worse disease-free survival ($P = 0.15$) than that of patients with aberrant expression of RAR-β (12). Aberrant expression of RAR-β was defined as expression in $<$10% of all cancer cells assayed in the tumor or no expression. Errant expression of RAR-β was defined as expression in $<$10% of all cancer cells assayed in the tumor or no expression. We are attempting to further understand the regulatory implications of these surprising findings.

The key enzyme involved in prostanoid synthesis from arachidonic acid is designated as COX (13). Two forms of this

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[3] The abbreviations used are: NSCLC, non-small cell lung cancer; COX-2, cyclooxygenase-2; ISH, *in situ* hybridization; RAR, retinoic acid receptor; RXR, retinoid X receptor.

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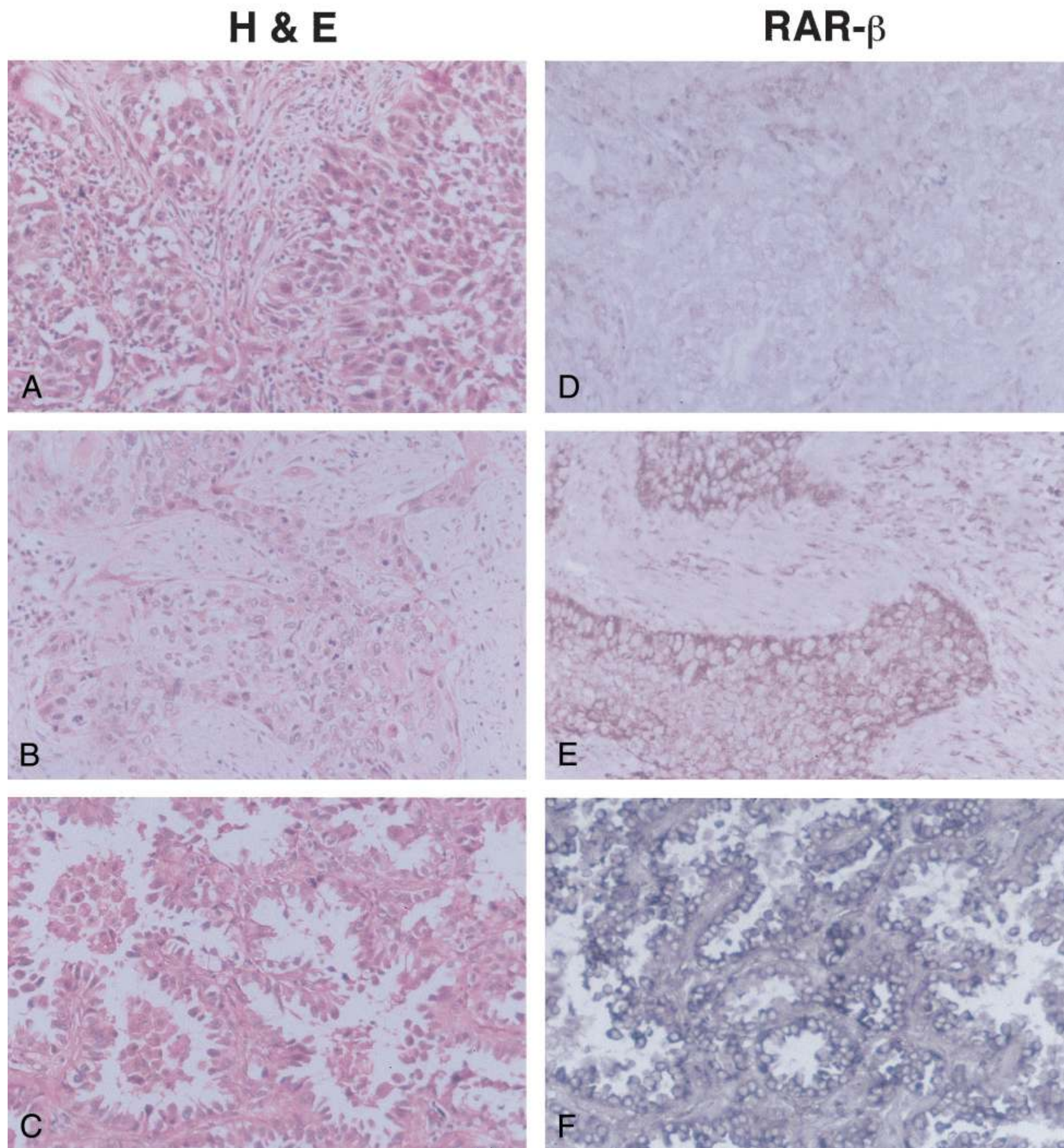

Fig. 1 Three different stage I lung cancers stained with H&E to demonstrate histology on the *left-hand side* (*A–C*) and *D–F* demonstrating staining with the RAR-β antisense riboprobe. The antisense riboprobe to RAR-β mRNA reveals strong staining in *F*, weak (<10% staining) in *E*, and absent staining in the cytoplasm of the lung cancer cells of *D*.

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enzyme exist in the mammalian body, constitutive COX-1 and inducible COX-2. COX-2 is responsible for many inflammatory processes and is up-regulated by various tumor promoters and growth factors. It is overexpressed in lung, head and neck, colon, and pancreatic cancers among other tumors (13–15). In several previous studies, the prognostic significance of elevated COX-2 expression in primary lung adenocarcinomas was evaluated (14–16) but none had correlated COX-2 with the expression of nuclear retinoid receptors. Furthermore, virtually all of these previous studies assayed for COX-2 protein using immunohistochemical methods, which were designed to detect protein expression in contrast to the *in situ* hybridization method used in this report to detect COX-2 expression at the mRNA level. Previous reports and our recent data indicated that retinoic acid

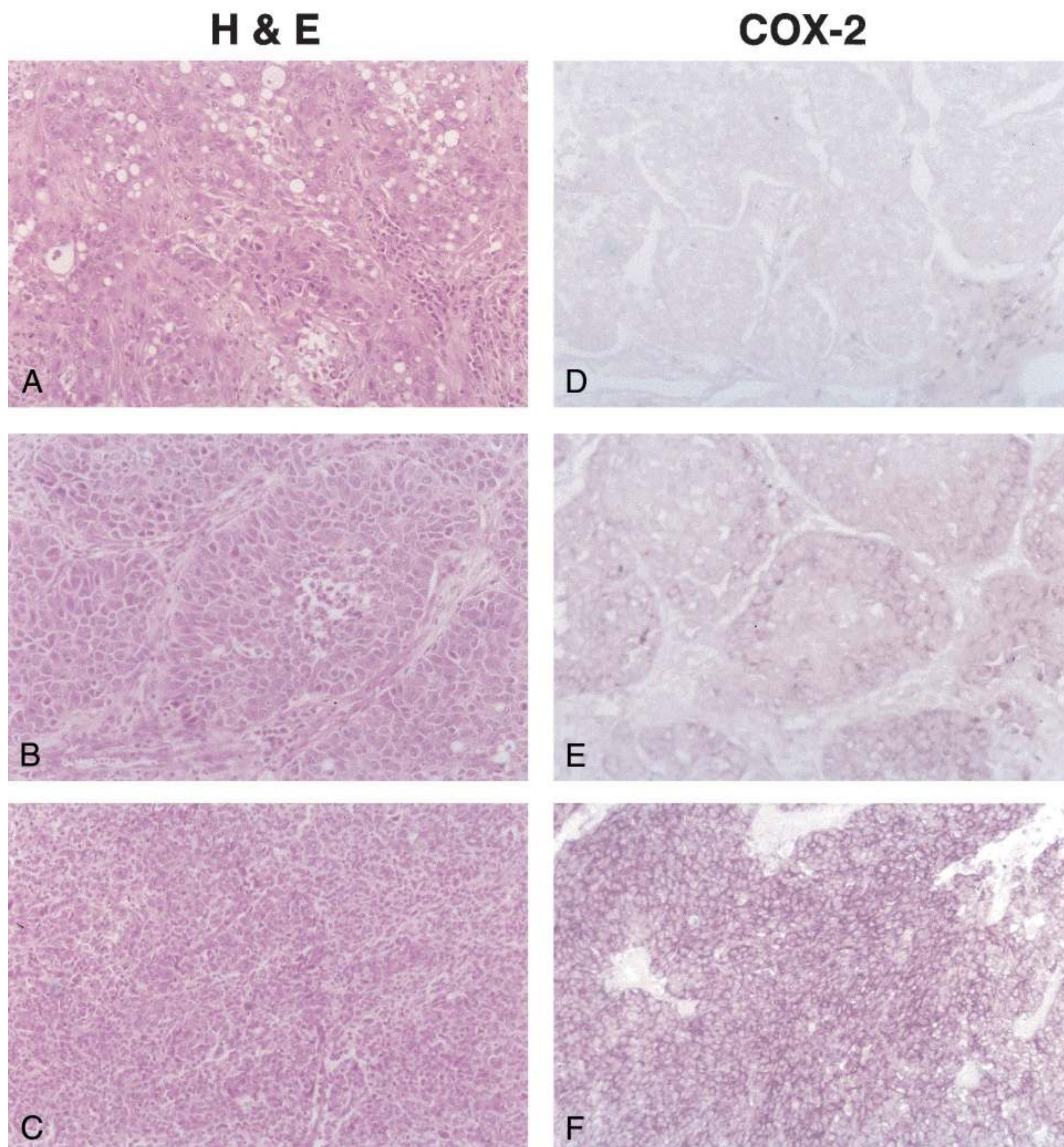

Fig. 2 Similarly to Fig. 1, three different stage I lung cancers stained with H&E to demonstrate histology on the *left-hand side* (*A–C*) and *D–F* demonstrate staining with the COX-2 antisense riboprobe. The antisense riboprobe to RAR-β mRNA-COX-2 reveals no staining in *D*, weak (<10%) in *E*, and strong staining in the cytoplasm of the lung cancer cell of *F*.

can reduce COX-2 expression in head and neck and esophageal cancers. RAR-β is among the most frequently altered RARs in head and neck (17) and non-small cell lung carcinogenesis (18, 19). It has been shown to be a retinoic acid-inducible gene. One therefore presumes that COX-2 may be downstream of RAR-β, and evidence exists to support these assumptions (20). In this context, we evaluated the expression of COX-2 in NSCLC and correlated its expression with that of RAR-β.

MATERIALS AND METHODS

Five hundred ninety-five consecutive patients with stage I NSCLC underwent definitive surgical resection, defined as a lobectomy or a pneumonectomy, from 1975 to 1990 at The University of Texas M. D. Anderson Cancer Center. We retrospectively examined 185 cases in which adequate tissue samples and data from a follow-up period of more than 5 years were

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available. The patient population was identified through a search of the Tumor Registry Database maintained by the Department of Medical Informatics at M. D. Anderson Cancer Center. One hundred sixty-three cases had both adequate intratumoral specimens and >5 years of verifiable follow-up. Median follow-up on this cohort was 9 years. Twenty-two cases were removed from the analysis because of inadequate tumor specimens (13 cases), assaying negatively for various controls including RXR-α (7 cases), and unverifiable follow-up (2 cases). The seven cases were negative for RXR-α, RAR-β, and COX-2, leading to concern for possible mRNA degradation in these samples. Survival status was verified and updated from tumor registry records as of July 1, 1999. All available tissue blocks for each patient were reviewed for the presence of tumor by a thoracic pathologist (B. L. K.). Sections (4-μm thick) were cut from specimen blocks using a microtome. To prevent RNA degradation in the tissues during sectioning, we used water treated with diethylpyrocarbonate (Sigma, St. Louis, MO) and glass slides that were pretreated by immersion in 70% ethanol-1% HCl for 3 days, washed with 70% ethanol, rinsed in distilled water, and baked at 180°C for at least 4 h. The slides were further coated with poly-lysine (Sigma) and then air dried overnight.

ISH. We used ISH to evaluate mRNA expression of both RAR-β and COX-2. To date, there are three major resources that provide anti-COX-2 antibodies, *i.e.*, Oxford Biochemical Research (Oxford, MI), Transduction Laboratories (Lexington, KY), and Cayman Chemicals (Ann Arbor, MI), and the majority of recent publications used these antibodies for COX-2 work (13–16). We have tried all three antibodies, and Western blotting showed at least two to three bands at the optimal conditions in these tissues. Immunohistochemistry using these antibodies showed that both epithelial cells and stromal cells are positive or both types of cells are negative, indicating either staining heterogeneity or artifact. Therefore, it was reasonable for us to choose *in situ* hybridization techniques to analyze COX-2 expression instead of using immunohistochemistry, particularly since we have extensive experience in performing ISH techniques. Although detection of protein expression is, in general, preferable to that of mRNA, qualified antibody will be required for immunohistochemistry or Western blotting.

We evaluated material from 163 patients for RAR-β and from 160 patients for COX-2 expression, with material from 158 patients being assessed for both molecular prognostic variables. RAR-β and RXR-α mRNAs were detected in tissue sections using nonradioactive ISH with digoxigenin-labeled antisense riboprobes, exactly as described by Xu *et al.* (17) (Fig. 1). RXR-α, which we found to be present in 95.1% of our prior stage I NSCLC (12), was used as a control to exemplify for a lack of RNA degradation. The reason for using RXR-α as a control for intact RNA in tissues is because all 70 cases of NSCLC in normal lung tissue expressed RXR-α in our initial study (18). If cases were positive for COX-2 and RXR-α negative, we also included them in the analyses. Cases that were negative for both COX-2 and RXR-α were not analyzed for survival. We used identical techniques to evaluate mRNA expression of COX-2, also using a digoxigenin-labeled antisense riboprobe to COX-2, and used ISH to assess COX-2 expression. Furthermore, we evaluated COX-2 expression using a descriptive statistic for interpreting COX-2 mRNA expression by ISH. Tissue samples were scored as 0, absent; 1, weak; 2, moderate; or 3, strong in terms of intratumoral COX-2 expression (Fig. 2). All slides were reviewed by two independent pathologists (B. L. K. and X-C. X.) and one medical oncologist (F. R. K.). The analysis was blinded, and the reproducibility of scoring was determined on a second occasion when the slides were reviewed by the same three individuals in a blinded fashion.

Table 1 Correlation of Cox-2 (+, −)[a] and RAR$^-$ (+s, +w/−)

	COX-2 (+)	COX-2 (−)	Total
RAR-β (+s)	29	11	40
RAR-β (+w/−)	65	53	118
Total	94	64	158[b]

[a] +, +s, +w, − correspond to positive, strongly positive, weakly positive, and negative in the staining of ISH.

[b] Association between COX-2 and RAR-β expression ($P = 0.053$).

Statistics. Overall survival and disease-free survival rates were calculated using the Kaplan-Meier method, and the log rank test was used to compare the rates between various COX-2 expression groups. χ^2 was applied to test the association between two categorical variables. Two-sided *P* values were calculated.

RESULTS

Of the 185 cases that we had initially assayed for mRNA expression, 160 cases had ≥5-year follow-up, adequate intraspecimen tumor, and positive expression of either RXR-α or COX-2. Of these 160 cases assayed for COX-2 expression by ISH, 3 had strong expression, 69 had intermediate expression, 24 had weak expression, and 64 had no expression of COX-2. Expression of COX-2 correlated with RAR-β expression ($P = 0.053$, Table 1). Of 160 cases, 62 were adenocarcinoma and 70 were squamous cell cancers and 28 were other types of cancers. If one evaluates survival for the patient population by assaying COX-2 expression, positive *versus* negative, there is a trend favoring the COX-2-negative patients (Fig. 3*A*, $P = 0.106$). However, when one subcategorizes COX-2 expression into strongly positive (≥30% COX-2 cytoplasmic staining), intermediately positive (≥10% COX-2 positive, but <30%), weakly positive (≤10%), and negative, striking differences emerge. Here, COX-2 expression was associated with worse overall survival (Fig. 3*B*, $P = 0.001$). COX-2 expression was also associated with worse disease-free survival when the COX-2 expression was classified as positive *versus* negative (Fig. 4*A*, $P = 0.111$) or more particularly when one subcategorizes COX-2 expression into strong, intermediate, weak, or absent (Fig. 4*B*, $P = 0.022$). The percentage of tumors that were COX-2 positive was not significantly different between the lung adenocarcinomas 37 of 62 or 59.7%) or the squamous cell cancers (45 of 70 or 64.2%; $P = 0.59$). If one subcategorizes COX-2 expression and compares COX-2 expression between 62 adenocarcinomas and 70 squamous cell carcinomas (28 others), there appears to be even less of a difference (Table 2, $P = 0.947$). Positive COX-2 expression correlates with a worse disease-free survival in the squamous cell cancers ($P = 0.013$; data not shown) and a trend toward worse disease-free survival

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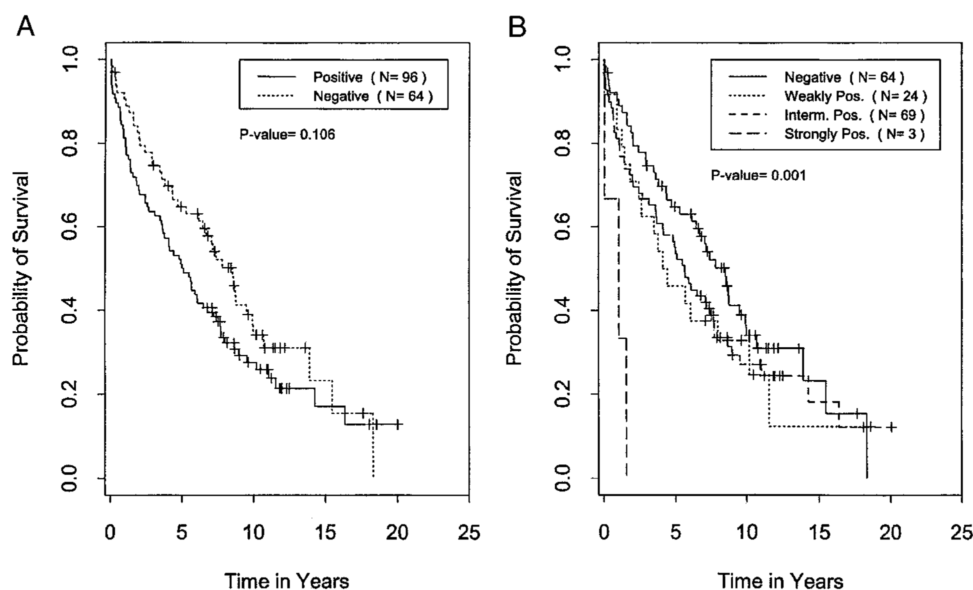

Fig. 3 *A*, survival for all 160 patients is correlated with COX-2 expression (negative *versus* positive). As can be seen, there is a trend for a better overall survival for the COX-2-negative group ($P = 0.106$). *B*, this figure assesses overall survival by COX-2 status. Negative indicates no expression of COX-2, weak expression is between 0 and 10% of cancer cells staining positive, intermediate expression is between 10 and 30%, and strong expression is ≥30%. As indicated, there is a statistically significant prognostic value for COX-2 expression ($P = 0.001$).

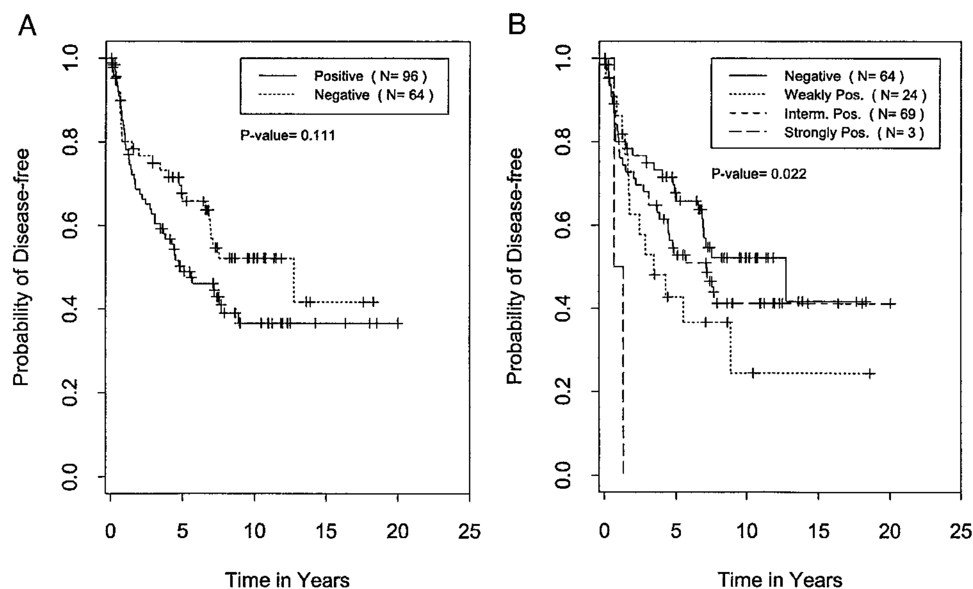

Fig. 4 *A*, this figure shows disease-free survival by COX-2 status. As in Fig. 3, patients with strong COX-2 expression do particularly poorly, but the difference is not statistically significant ($P = 0.111$). *B*, when one evaluates disease-free survival and subcategorizes COX-2 expression into strong, intermediate, weak, or absent, the difference is statistically significant ($P = 0.022$).

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($P = 0.144$; data not shown). The same trend was found in adenocarcinomas for the overall survival but the association was weaker (data not shown).

The median survival times for the strong, weak, or intermediate and null COX-2 expressors were 1.04, 5.50, and 8.54 years, respectively. Most striking was the extremely poor outcome among the three patients whose samples showed strong COX-2 expression. All three patients with strong COX-2 expression had positive expression of RAR-β in their tumors as well, and all died within 19 months of diagnosis of stage I NSCLC. Figs. 3*B* and 4*B* reflect the significantly poor overall and disease-free survival times for patients whose samples had strongly positive COX-2. All three patients developed metastases from their primary stage I NSCLC within 16 months. The weak or intermediate positive group had an intermediate prognosis, and patients with absent COX-2 had the best overall outcome (Figs. 3 and 4).

We further attempted to correlate between COX-2 expression and several known molecular prognostic factors, including expression of vascular endothelial growth factor, basis fibroblast growth factor, mean vascular density, interleukin 8 expression, and *k-ras* gene mutational status. None of these markers correlated independently with COX-2 expression, with the correlation coefficients for vascular endothelial growth factor *(P* = 0.16), basic fibroblast growth factor ($P = 0.77$), interleukin 8 ($P = 0.65$), mean vascular density ($P = 0.59$), and k-*ras* ($P = 0.25$) failing to attain significance when correlated with COX-2 expression. COX-2

Table 2 Frequency of COX-2 status (0–3) versus histology (ADENO, SQ)

Histology	COX-2 = 0	COX-2 = 1	COX-2 = 2	COX-2 = 3	Total
ADENO[a]	25	10	26	1	62
SQCC	25	11	32	2	70
Total	50	21	58	3	P = 0.947 (Fisher's exact test)

[a] ADENO, adenocarcinoma; SQCC, squamous cell carcinoma.

remained an independent prognostic expression factor in multivariate analysis.

DISCUSSION

Our results continue to define a subpopulation with poor prognosis among patients with stage I NSCLC as a group with strong expression of RAR-β and overexpression of COX-2. Most strikingly, those patients with particularly strong expression of COX-2 and RAR-β (we saw only 3 patients in our study of 158 patients for whom both prognostic markers could be evaluated) all died of metastatic disease within 19 months. All developed their metastases <16 months after surgical resection of what were true surgical stage I NSCLCs. This outcome is particularly poor, as the 5-year survival rate after surgery has been reported to be between 60 and 80% for patients with surgical stage I disease (3–6) and their 2-year survival rate is >80%. The molecular features of these three cases are interesting in that although all three cases have strong expression of COX-2 and strong or intermediate expression of RAR-β, only two of three had k-*ras* mutations detectable by PCR-Introduced Restriction with Enrichment for Mutation Alleles (PIREMA). Furthermore, the group of patients with strong RAR-β positivity was significantly more likely to have COX-2 expression compared with the RAR-β-negative group (P = 0.053). This is unexpected based on cell line data, as retinoids are known to down-regulate expression of COX-2 in cancer cell lines that overexpress COX-2 (20).

In a previous evaluation of the prognostic significance of COX-2 in lung cancer, Achiwa *et al.* (16) evaluated the expression of COX-2 by immunohistochemistry in surgically resected adenocarcinomas of the lung. Their data indicate that an increase in COX-2 expression may be clinically significant for the prognosis of patients undergoing surgical resections, particularly of stage I NSCLC. In their study, elevated COX-2 expression was associated with shortened survival time of patients with stage I disease (P = 0.034). In the study of Achiwa *et al.* (16), 57 patients were COX-2 positive and 24 were COX-2 negative. If we evaluate our data for COX-2 expression simply by assaying for COX-2 status (positive or negative), there is a trend for a worse overall survival for COX-2-positive patients (Fig. 3*A*, P = 0.106). Our data indicated that shortened disease-free survival was also seen in stage I squamous cell cancers, if one simply evaluated COX-2-positive *versus* COX-2-negative squamous cell cancers (P = 0.013, data not shown). Achiwa *et al.* (16) showed a higher likelihood of COX-2 expression by immunohistochemistry for adenocarcinomas as opposed to squamous cells cancers. Our data, assaying mRNA by ISH, does not confirm this finding.

This was also the first study to assay COX-2 mRNA expression using ISH techniques, as opposed to the more widely used immunohistochemical assays for COX-2 protein. In our experience, ISH was more sensitive and specific for the detection and stratification of COX-2 mRNA than immunohistochemical methods to detect COX-2 protein (data not shown). Because of the very high background that can be seen when using immunohistochemical staining, several laboratories are using quantitative PCR to assay for COX-2. Our expertise with ISH and our long-standing interest in evaluating COX-2 mRNA and comparing it with RAR-β mRNA led us to prefer this technique.

Our attempt to better understand the mechanistic underpinnings of lung cancers has led us to evaluate the connection between the COX-2 and retinoid pathways, mindful of the fact that cell line data often do not translate into well-defined clinical-pathological observations. One possible connection between COX-2 and the retinoid pathways may be via the *ras*/*erk* signal transduction pathway. It is known that mutations in the k-*ras* gene are found in ~30% of lung adenocarcinomas and are associated with an inferior overall survival rate, irrespective of treatment (7). Human NSCLC cell lines with mutations in k-*ras* have high expression levels of COX-2 and inhibition of *ras* activity in these cell lines has been shown to decrease COX-2 expression (21). Furthermore, rat intestinal epithelial cells and fibroblasts transfected with h-*ras* overexpress COX-2, but inhibitors of *erk* block this response (22). We evaluated the *ras* gene status of these tumors by the PIREMA method and attempted to correlate with both the COX-2 and RAR-β status of the tumor. Interestingly, no correlation was seen in our tumors between k-*ras* mutational status and either COX-2 expression or RAR-β expression (P = 0.58 and P = 0.25, respectively).

Further data has indicated that RAR-β is capable of inhibiting transformation via the *ras* signaling pathway by down-regulation of the activator protein 1 transcription complex (23). Mestre *et al.* (20) demonstrated that retinoids (9-*cis*-retinoic acid, 13-*cis*-retinoic acid, and retinyl acetate) suppress both basal levels of COX-2 and epidermal growth factor-mediated induction of COX-2 protein . The link between COX-2 expression and down-regulation by retinoids led us to evaluate COX-2 in our patients with stage I disease in whom we had previously found, to our surprise, that strong expression of RAR-β conferred a poor prognosis (12). In other words, cell line data would predict that functional expression of RAR-β and its up-regulation by retinoids should have resulted in down-regulation of COX-2 expression, a finding in contrast to our study findings.

At this point, the biological significance of RAR-β positivity and COX-2 expression will require further studies in cell lines to better understand their interaction in NSCLC. While we speculate that RAR-β signaling is abrogated in these NSCLC tumors, and this may be reflected by the high COX-2 expression, further mechanistic studies will be vital to our understanding of this phenomenon. *ras* mutation can lead to loss of retinoid signaling via their receptors (22). Although we did not see a strong correlation between k-*ras* mutations and either COX-2 or RAR-β, the interaction between these pathways remains intriguing and potentially important.

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In evaluating the three patients who all had 3+ COX-2 expression and intermediate to strong RAR-β expression for multiple prognostic markers, including *ras* gene status, we were unable to determine any distinguishing clinical or pathological features that made them more likely to suffer the poor outcome that they experienced. Their tumors were neither especially large nor their other features more suggestive of a higher metastatic potential. The possible reasons for the correlation of RAR-β positivity with a poor outcome in our original study are unclear to us, as we indicated in our discussion of the above results (12). The role of RAR-β, whether or not it is functional, and the associated downstream events remain equally unclear at this time. In our study, COX-2 expression correlated directly with RAR-β expression and poor prognosis. Understanding this COX-2/RAR-β dysregulation in NSCLC may be important to the development of prevention and therapy interventions with retinoids and/or COX-2 antagonists in this patient population (24–26).

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REFERENCES

1. Greenlee, R. T., Murray, T., and Bolden, S., and Wingo, P. A. Cancer statistics. CA Cancer J. Clin., *50:* 7–33, 2000.

2. Ginsberg, R. J., Kris, M. G., and Armstrong, J. G. Cancer of the lung. *In:* V. T. DeVita, S. Hellman, and S. A. Rosenberg (eds.), Cancer: Principles and Practice of Oncology, Ed. 4, pp. 673–722. Philadelphia: Lippincott, 1994.

3. Martini, N., McCaughan, B. C., and McCormack, P., et al. Lobectomy for stage I lung cancer. *In:* C. F. Kittle (ed.), Current Controversies in Thoracic Surgery, pp. 171–174. Philadelphia: Saunders, 1986.

4. Williams, D. E., Pairolero, P. C., Danis, C. S., Bernatz, P. E., Payne, W. S., Taylor, W. F., Uhlenhopp, M. A., and Fontana, R. S. Survival of patients surgically treated for stage I lung cancer. J. Thorac. Cardiovasc. Surg., *82:* 70–76, 1981.

5. Teramachi, M., Miyamoto, N., Sasaka, T., Makamura, T., and Kitamuta, F. Results of surgery in non-small cell lung cancer. Bull. Chest Dis. Res. Inst. Kyoto Univ., *23:* 48–53, 1990.

6. Naruke, T., Goya, T., Tsuchiya, R., and Suemasu, K. Prognosis and survival in resected lung carcinoma based on the new international staging system. J Thorac. Cardiovasc. Surg., *96:* 440–447, 1988.

7. Slebos, R. J., Kibbelaaar, R. E., Dalesio, O. Kooistra, A., Stam, J., Meijer, C. J., Wagenaar, S. S., Vanderschueren, R. G., van Zandwijk, N., and Mooi, W. J. K-*ras* oncogene activation as a poor prognostic marker in adenocarcinoma of the lung. N. Engl. J. Med., *323:* 561–565, 1990.

8. Lee, J. S., Ro, J. Y., Sahin, A. A., Hong, W. K., Brown, B. W., Mountain, C. F., and Hittelman, W. F. Expression of blood-group antigen A—a favorable prognostic factor in non-small cell lung cancer. N. Engl. J. Med., *324:* 1084–1090, 1991.

9. Pezzella, F., Turley, H., Kuzu, I., Tungekar, M. F., Dunnill, M. S., Pierce, C. B., Harris, A., Gatter, K. C., and Mason, D. Y. bcl-2 protein in non-small-cell lung carcinoma. N. Engl. J. Med., *329:* 690–694, 1993.

10. Pastorino, U., Andreola, S., Tagliabue, E., Pezzella, F., Incarbone, M., Sozzi, G., Buyse, M., Menard, S., Pierotti, M., and Rilke, F. Immunocytochemical markers in stage I lung cancer: relevance to prognosis. J. Clin. Oncol., *15:* 2858–2865, 1997.

11. Kwiatkowski, D. J., Harpole, D. H. Jr., Godleski, J., Herndon, J. E., II, Shieh, D. B., Richards, W., Blanco, R., Xu, H. J., Strauss, G. M., and Sugarbaker, D. J. Molecular pathologic substaging in 244 stage I non-small-cell lung cancer patients: clinical implications. J. Clin. Oncol., *16:* 2468–2477, 1998.

12. Khuri, F. R., Lotan, R., Kemp, B. L., Woo, H., Lippman, S. M., Fen, L., Lee, J. J., Apr, B., Cooksley, C., Chang, E., Walsh, G., Lee, J. S., Hong, W. K., and Xu, X-C. The role of RAR-β as a prognostic indicator in stage I non-small cell lung cancer. J. Clin. Oncol., in press, 2001.

13. Taketo, M. M. Cyclooxygenase-2 inhibitors in tumorigenesis (part I). J. Natl. Cancer Inst., *90:* 1520–1529, 1998.

14. Hida, T., Yatabe, Y., Achiwa, H., Muramatsu, H., Kozaki, K-I., Nakamura, M., Ogawa, M., Mitsudomi, T., Sugiura, T., and Takahashi, T. Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinoma. Cancer Res., *58:* 3761–3764, 1998.

15. Wolff, H. Saukkonen, K., Anttila, S., Karjalainen, A., Vainio, H., and Ristimaki, A. Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res., *58:* 4997–5001, 1998.

16. Achiwa, H., Yatabe, Y., Hida, Y., Kuroshi, T., Kozaki, K-I. Nakamura, S., Ogawa, M., Sugiura, T., Mitsudomi, T., and Takahashi, T. Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinoma. Clin. Cancer Res., *5:* 1001–1005, 1999.

17. Xu, X. C., Ro, J. Y., Lee, J. S. Shin, D., 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.

18. Xu, X-C., Sozzi, G., Lee, J. S., Lee, J. J., Pastorino, U., Pilotti, S., Kurie, J. M., Hong, W. K., and Lotan, R. Suppression of retinoic acid receptor β in non-small-cell lung cancer in vivo: implications for lung cancer development. J. Natl. Cancer Inst., *89:* 624–629, 1997.

19. Xu, X-C., Lee, J. S., Lee, J. J., Morice, R., Liu, X., Lippman, S. M., Hong, W. K., and Lotan, R. Nuclear retinoid acid receptor β in bronchial epithelium of smokers before and during chemoprevention. J. Natl. Cancer Inst., *91:* 1317–1321, 1999.

20. Mestre, J. R., Subbaramaiah, K., Sacks, P. G., Schantz, S. P., Tanabe, T., Inoue, H., and Dannenberg, A. J. Retinoids suppress epidermal growth factor-induced transcription of cyclooxygenase-2 in human oral squamous carcinoma cells. Cancer Res., *57:* 2890–2895, 1997.

21. Heasley, L. E., Thaler, S., Nicks, M., Price, B., Skorecki, K., and Nemenoff, R. A. Induction of cytosolic phospholipase A_2 by oncogenic *ras* in human non-small cell lung cancer. J. Biol. Chem., *272:* 14501–14504, 1997.

22. Sheng, H., Williams, C. G., Shao, J., Liang, P., DuBois, R. N., and Beauchamp, R. D. Induction of cyclooxygenase-2 by activated Ha-*ras* oncogene in Rat-1 fibroblasts and the role of mitogen-activated protein kinase pathway. J. Biol. Chem., *273:* 22120–22127, 1998.

23. Zhou, X. F., Shen, X. Q., and Shemshedini, L. Ligand-activated retinoic acid receptor inhibits AP-1 transactivation by disrupting c-jun/c-fos dimerization. Mol. Endocrinol., *13:* 276–285, 1999.

24. Hida, T., Leyton, J., Makheja, A. N., Ben-Av, P., Hla, T., Martinez, A., Mulshine, J., Malkani, S., Chung, P., and Moody, T. W. Non-small cell lung cancer cyclooxygenase activity and proliferation are inhibited by non-steroidal anti-inflammatory drugs. Anticancer Res., *18:* 775–782, 1998.

25. Hong, W. K., and Sporn, M. B. Recent advances in chemoprevention of cancer. Science (Wash DC), *278:* 1073–1077, 1997.

26. Lippman, S. M., Lee, J. J., and Sabichi, A. L. Cancer chemoprevention: progress and promise. J. Natl. Cancer Inst., *90:* 1514–1528, 1998.

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