Sex-Specific Survival Advantage with Parathyroid Hormone–Related Protein in Non–Small Cell Lung Carcinoma Patients

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Abstract

Purpose: Parathyroid hormone–related protein (PTHrP) is commonly expressed in non–small cell lung carcinomas (NSCLC). Expression of the protein could have implications for progression of the disease because it regulates cancer cell growth, apoptosis, and angiogenesis. However, its relationship with survival has not been evaluated in a large-scale investigation.

Experimental Design: PTHrP expression was assessed in paraffin-embedded tumor samples from 407 patients with NSCLC by immunohistochemistry. A pathologist unaware of the clinical history classified specimens as PTHrP positive or PTHrP negative. The log-rank test was used to compare survivals of PTHrP-positive and PTHrP-negative groups, and Cox regression was used to adjust for additional covariates.

Results: Median survival was 55 versus 22 months ($P < 0.001$) in female patients with and without tumor PTHrP, respectively. Male survival was 38 months independent of PTHrP status. Stage, histology, age, and smoking history were also associated with increased longevity. PTHrP remained a significant predictor of survival for female patients after controlling for stage, histology, and age.

Conclusions: In this study, PTHrP expression was associated with a survival advantage in female patients. Additional investigations must be done to ascertain whether the result is reproducible and independent of potential confounding covariates. Sex-dependent effects of PTHrP in lung cancer would open new avenues of research into the role of sex in cancer progression.

Parathyroid hormone–related protein (PTHrP) was discovered as the factor responsible for hypercalcemia of malignancy. As the name implies, PTHrP shares structural similarities with PTH, specifically in the NH_2-terminal 34 amino acids. Consequently, PTHrP 1-34 binds to the same receptor as PTH 1-34, the type 1 PTH/PTHrP receptor (PTH1R), and duplicates the effects of PTH 1-34 in tissues that bear the receptor, including causing hypercalcemia. Hypercalcemic effects are mediated through decreases in calcium excretion in kidney and increases in calcium release from bone. It is less widely appreciated that PTHrP also exerts effects direct effects on cancer cells that could be important in the pathology of malignancy.

Direct actions of PTHrP on cancer cells are frequently related to cell growth or life cycle. For example, the protein alters the rate of proliferation in many cancer cell types (1–4) and can have regulatory effects on the propensity to undergo apoptosis (5–7). PTHrP also modulates integrin (8–11) and metalloproteinase expression (12) in various cancer cells and inhibits angiogenesis (13). Effects of this type would give PTHrP the capacity to regulate tumor progression, alter sensitivity to therapy, modulate metastasis, and contribute to the pathogenesis of cancer in ways in addition to causing hypercalcemia. In fact, PTHrP affects disease progression in several animal models of malignancy and seems to be a prognostic factor in some types of cancer in humans (14–19).

Because roughly two thirds of non–small cell lung cancers (NSCLC) express PTHrP regardless of histology (20), the protein could be important in the pathophysiology of the disease beyond its role in causing hypercalcemia. A large body of work has evaluated the role of PTHrP in lung cancer–induced hypercalcemia (21), but few investigations have probed its role in lung cancer progression or survival. An animal study from our laboratory suggested that PTHrP expression could hinder lung cancer growth (1). Hence, the goal of this project was to conduct a large patient study to determine whether survival of patients with NSCLC varied based on tumor PTHrP expression and to examine the influence of PTHrP with other factors known to affect patient life span in lung cancer.

Materials and Methods

Study population. These investigations were done after approval by the University of California San Diego (UCSD) Institutional Review Board and in accordance with an assurance filed with and approved by the Department of Health and Human Services. The Institutional Review

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Board waived the requirement for informed consent because the study used preexisting pathologic material and records and met all HIPAA and Institutional Review Board requirements for such a waiver. The patients were drawn from three sources, the VA San Diego Healthcare System (VASDHS), the University of California Medical Center (UCSD), and a NSCLC tissue microarray (Cytomyx US, Boston, MA). The study included all the patients from VASDHS and UCSD between January 1983 and December 2001 who had surgery for treatment of their primary NSCLC and usable tumor blocks in the pathology department tissue archive. The samples in the Cytomyx tissue microarray set were surgical or biopsy specimens of patients with NSCLC obtained from a number of other hospitals in the period of 1986 to 2001. The median follow-up times among patients who did not die were 44, 29, and 49 months for VASDHS, UCSD, and Cytomyx patients, respectively.

Specimen preparation. Specimens were fixed in neutral buffered formalin (10% v/v) and embedded in paraffin through a graded butanol series. Sections (5 μm) were cut, deparaffinized through a xylene to ethanol series, rehydrated, and mounted on charged glass slides. In some patients, sections were obtained from blocks of uninvolved lung distant from the cancer. Tissue arrays were prepared commercially (Clinomics, Watervliet, NY, company acquired by Cytomyx US). A board-certified pathologist examined H&E-stained slides of tumor blocks that had been prepared in the same fashion and selected areas of tumor that were fixed well and devoid of preparation artifacts. Areas were marked on the matched block, and 0.6-mm tissue cores were cut from the marked area. The tissue array was constructed with a Beecher Instruments arraying instrument (Sun Prairie, WI). It contained two independent tumor specimens from each NSCLC patient, a spot from an uninvolved area of normal lung distant from the tumor in most of the NSCLC patients, noncancerous lung from additional patients without cancer, and samples of normal kidney, prostate, and colon. Arrays were prepared on slides as 5-μm sections. Before releasing the material for research use, the pathologist reviewed an H&E-stained slide of the array to verify the presence of tumor, the quality of the tissue, and the absence of artifacts.

Immunohistochemistry and histology. PTHrP was stained with an immunohistochemical technique as described previously (22, 23). Antigen retrieval techniques were not employed. The mouse monoclonal primary antibody directed against PTHrP 109-141, 9H7 (22, 23), was used at a concentration of 10 μg/mL for 1 hour at room temperature. Immunoreactivity was developed with a standard avidin-biotin complex alkaline phosphatase using Vector Red (kit SK-5100, Vector Laboratories, Burlingame, CA) as the chromogen and a 0.125% methyl green counterstain. Staining controls with each run included sections in which primary antibody was omitted and an interassay standard, a known PTHrP-positive lung carcinoma, for quality control. PTHrP immunoreactivity was negligible if primary antibody was omitted or preadsorbed with a 100-fold excess of antigen (data not shown). Staining of the interassay control was maintained at a constant level from one staining run to the next by titrating and adjusting the concentration of primary antibody periodically as indicated by changes in staining of the quality control slides. A serial section from each cancer was stained with H&E to evaluate section quality and cancer histology.

Slide evaluation. Sections were reviewed for the presence of tumor and evaluated for PTHrP immunoreactivity and distribution (cytoplasmic or nuclear) by a practicing surgical pathologist (A.L.) with extensive experience interpreting immunohistochemical stains and knowledge of

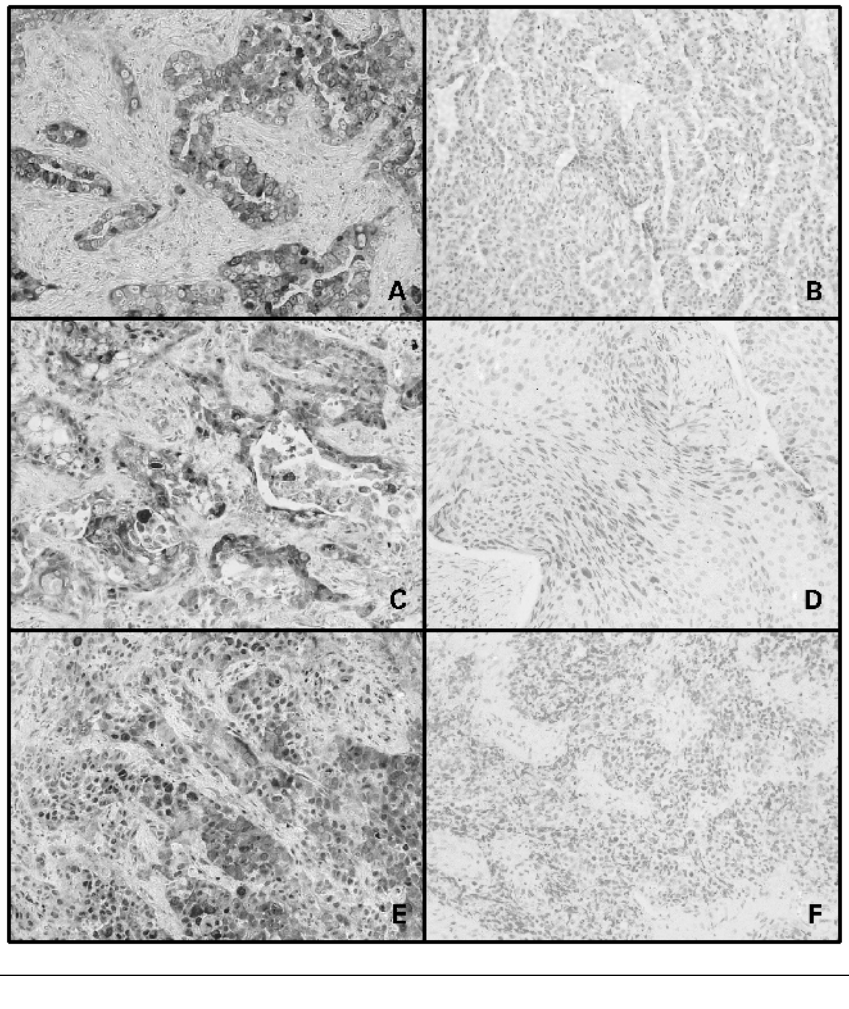

Fig. 1. PTHrP-positive and PTHrP-negative NSCLCs. The micrographs are positive and negative examples of adenocarcinoma (*A* and *B*), squamous carcinoma (*C* and *D*), and large cell carcinoma (*E* and *F*). Immunoreactivity was absent in PTHrP-negative tissues or if primary antibody was omitted (data not shown). Procedures were done with close quality assurance using multiple negative controls and interassay controls. A pathologist blinded to the clinical data evaluated staining.

Table 1. Demographic data and lung cancer information divided by institution

	VASDHS	UCSD	Cytomyx	Total
Total numbers	104 (100)	221 (100)	82 (100)	407 (100)
Stage*				
I	63 (61)	98 (44)	65 (79)	226 (55)
II	11 (11)	18 (8)	15 (180)	44 (11)
III	13 (12)	53 (24)	2 (3)	68 (16)
IV	5 (5)	15 (7)	0	20 (6)
Unknown	12 (11)	37 (17)	0	49 (12)
Histology†				
Adeno	63 (60)	93 (42)	36 (44)	192 (47)
Squamous	34 (33)	113 (51)	32 (39)	179 (44)
Large cell	7 (7)	15 (7)	12 (15)	34 (8)
Unknown	0	0	2 (2)	2 (1)
Smoking				
Yes	95 (91)	186 (84)	—	281 (69)
No	4 (4)	20 (9)	—	24 (6)
Unknown	5 (5)	15 (7)	82 (100)	102 (25)
Sex				
Male	104 (100)	124 (56)	54 (66)	282
Female	0	97 (44)	27 (33)	124
Mean age ± SE (y)	67 ± 1	62 ± 1	67 ± 1	64 ± 1

NOTE: Data are number (% column total) unless otherwise specified. Sex was unknown for one Cytomyx patient.
*$P < 0.001$ among the three institutions.
†$P < 0.01$ among the three institutions.

the pitfalls of this technique. The quality of the positive control for each run was assessed for several criteria: intracellular staining without stain lake formation, minimal noncellular debris was minimal, and absence of indiscriminate background staining outside of the boundaries of the cells. Some variation of staining strength within the positive control slide was expected and was reassuring that the stain was titrated properly (not overly strong or weak). Runs and individual cases underwent repeat staining if there was evidence of significantly uneven or patchy staining (suggesting inadequate coverage of the slide with reagent), excessive deposition of noncellular debris, formation of stain lakes, or tissue falling away from the slide. Areas of tissue necrosis were avoided in the interpretations to reduce interpretation of nonspecific staining.

The evaluator was unaware of the patient clinical data. Specimens were considered positive if PTHrP immunoreactivity was present above background in any lung cancer cell. Each case was interpreted in direct comparison with its negative control. Any positive staining that was matched by staining on the negative control slide was not counted in the interpretation. Tissue microarray specimens were classified as PTHrP positive if either tissue spot from the same patient was positive.

Patient clinical data. Patient demographic and clinical data were collected from the VASDHS and UCSD Cancer Registry database, using CNEXT software (Public Health Institute) and from the medical record. Data included age, sex, race and ethnicity, date of diagnosis, smoking history, tumor stage, tumor histology, treatment, occurrence of distant metastasis after diagnosis, and survival status at the date of latest follow-up. Tumor histology was taken from the original pathology report and was based on standard surgical pathology diagnostic criteria as outlined by WHO. Stage was assigned based on the American Joint Committee on Cancer Criteria as of 2002 (24). Data on out-of-hospital mortality was obtained from the U.S. Social Security Administration and the State of California Death Registries. Pack-years of smoking, defined as the product of the patient's estimated average number of packs smoked per day and the years of smoking, was obtained from the clinical record, when available. The record generally did not provide the component data for pack-years, the packs per day or years of smoking.

Data analysis. Categorical data were tabulated as frequencies and percentages. Continuous data were reported as medians. Comparisons between groups were done by the Wilcoxon rank sum test for continuous variables or Fisher's exact test for categorical data. Kaplan-Meier curves were plotted by PTHrP status, positive or negative. Survival curves were compared using the log-rank test, and multivariate survival analysis was carried out using the Cox proportional hazards regression. Statistical analyses were done with StatView 5.0.1 (SAS Institute, Cary, NC), Stata 8.0 (College Station, TX), and R version 1.9 (R Foundation for Statistical Computing, Vienna, Austria; ref. 25).

Results

Our immunohistochemical stain clearly distinguished between PTHrP-positive and PTHrP-negative lung carcinomas. PTHrP-positive tumors contained bright reaction product localized with little background staining, whereas negative tumors showed no staining (Fig. 1). Lung carcinomas in ~67% of the patients contained PTHrP immunoreactivity. PTHrP expression was much less frequent in noncancerous lung than in tumors. In a matched set of malignant and nonmalignant tissue from 65 patients, 37 NSCLC specimens versus 11 uninvolved lung regions were PTHrP positive ($P < 0.001$). In another 34 lung specimens from patients without lung cancer, none stained for the protein ($P < 0.05$ versus uninvolved lung in patients with lung cancer).

Tables 1 and 2 summarize the demographics of our patient population, arranged either by institution or by tumor PTHrP status and sex. The majority of the patients had stage I carcinomas, and only a small percentage had stage IV tumors, as expected for patients selected for having a surgical specimen. The population from VASDHS and UCSD was 88% Caucasian, 7% Black, and 5% Asian and other backgrounds, reflecting the profile of patients in San Diego. Race, ethnicity, and smoking data were not available for Cytomyx patients. The three hospital groups differed with regard to distribution of stage and histology. The VASDHS population differed in being entirely male, but the distributions of sex did not differ between the other two patient sources. Smoking data was available only for VASDHS and UCSD and did not vary between these two groups.

Roughly two thirds of the patients had tumors that expressed PTHrP. Tumor PTHrP expression was similar for males (69%) and females (63%). Overall, sex, stage, histology, smoking history, and institution did not vary significantly between patient groups with PTHrP-negative and PTHrP-positive tumors. On the other hand, age varied between PTHrP-positive and PTHrP-negative tumor groups. Male and female patients with PTHrP-positive tumors were younger by 4 to 5 years, on average, than patients whose tumors showed no evidence of producing the protein. Although histology did not seem to vary overall with PTHrP status, a difference arose when the two sexes were analyzed separately. PTHrP-positive tumors in women were more likely to be adenocarcinomas ($P = 0.054$). Only 28% of the females in the Cytomyx group (11 of 27 patients) expressed PTHrP compared with 69% (67 of 97 patients) for UCSD females ($P < 0.05$). PTHrP expression was not significantly different comparing Cytomyx males with males in the other two groups.

Deaths occurred in 322 patients (79%) during the study period, 97 of 124 women (78%) and 224 of 282 men (79%).

Table 2. Demographic and lung cancer data by sex and tumor PTHrP expression

	All patients		Females		Males	
	PTHrP positive	PTHrP negative	PTHrP positive	PTHrP negative	PTHrP positive	PTHrP negative
Total no.	134 (100)	273 (100)	46 (100)	78 (100)	88 (100)	194 (100)
Stage						
I	73 (53)	153 (56)	27 (59)	48 (61)	46 (52)	104 (54)
II	14 (10)	29 (11)	2 (4)	3 (4)	12 (14)	26 (13)
III	30 (22)	39 (14)	8 (17)	9 (12)	22 (25)	30 (16)
IV	10 (7)	13 (5)	8 (17)	5 (6)	2 (2)	8 (4)
Unknown	11 (8)	39 (14)	5 (11)	13 (17)	6 (7)	26 (13)
Histology						
Adenocarcinoma	56 (42)	136 (50)	17 (37)*	46 (59)*	39 (44)	89 (46)
Squamous	65 (47)	114 (41)	24 (52)*	27 (35)*	41 (47)	87 (45)
Large cell	12 (8)	22 (8)	5 (11)*	5 (6)*	7 (8)	17 (8)
Unknown	1 (1)	1 (1)	0 (0)*	0 (0)*	1 (1)	1 (1)
Smoking						
Yes	88 (66)	193 (71)	25 (91)	52 (67)	63 (72)	141 (72)
No	4 (4)	18 (7)	3 (4)	11 (14)	3 (3)	7 (4)
Unknown	18 (30)	61 (22)	18 (5)	15 (19)	22 (25)	46 (24)
Smoker pack-years	50 (6, 160)	60 (2, 200)	50 (6, 100)	48 (2, 175)	52 (15, 160)	60 (3, 200)
Hospital						
VASDHS	28 (21)	76 (28)	0 (0)†	0 (0)†	28 (32)	76 (39)
UCSD	72 (54)	149 (55)	30 (65)†	67 (86)†	42 (48)	82 (42)
Cytomyx	34 (25)	48 (17)	16 (35)†	1 (14)†	18 (20)	36 (19)
Age‡ (y)	69 (43, 82)‡	64 (19, 89)‡	69 (49, 80)‡	65 (19, 89)‡	69 (43, 82)‡	64 (25, 87)‡

NOTE: Data are number (% column total) or median (range).
*$P = 0.054$, Fisher's exact test, excluding the unknown category.
†$P < 0.05$, Fisher's exact test, excluding the VASDHS group.
‡$P < 0.05$, PTHrP-positive versus PTHrP-negative patients.

PTHrP-negative and PTHrP-positive groups in all patients did not differ in survival, but large effects emerged when the data were separated by patient sex (Fig. 2). Median survival time in men was 38 months, regardless of PTHrP status. In contrast, the duration in women with PTHrP-positive NSCLC was 55 months compared with 22 months for women with PTHrP-negative tumors ($P < 0.001$). The hazard ratio for death was 0.51 (95% confidence interval, 0.34-0.77) for PTHrP-positive versus PTHrP-negative women ($P = 0.002$). In addition to PTHrP, stage, histology, and age were univariate predictors of mortality in females, but institution was not (Table 3). PTHrP remained an independent predictor ($P = 0.047$) of long survival in women after adjusting for the effects of these additional factors in a Cox regression model.

Smoking history was also a predictor for survival. The hazard ratio for a smoker was 3.2 (95% confidence interval, 1.37-7.57) compared with those never having smoked ($P = 0.007$) in female patients. Smoking history data were limited to a small number of female patients, and only 76 of the 124 females had a complete set of PTHrP, stage, histology, age, and smoking data. With this set of 76 female patients, all of the factors remained independent indicators of survival except PTHrP ($P = 0.29$). Survival curves using the data from all of the female patients with smoking history showed a tendency toward longer survival for PTHrP-positive tumors, whether they were

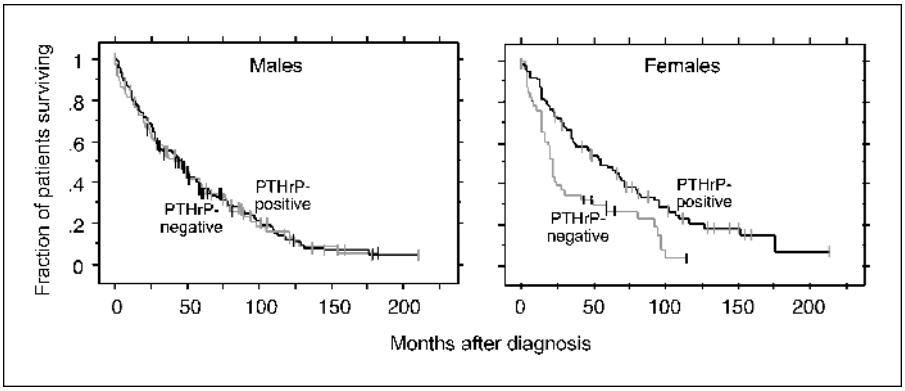

Fig. 2. Kaplan-Meier survival curves for male and female patients with NSCLC as a function of tumor PTHP. PTHrP expression was associated with prolonged survival in women ($P < 0.001$, log-rank) but not men. The data represent 194 PTHrP-positive males, 88 PTHrP-negative males, 78 PTHrP-positive females, and 46 PTHrP-negative females. Patients who were alive at their last follow-up were censored from analyses of later survival times. Points when patients were censored are indicated on the curves by vertical tick marks.

Table 3. Analysis of factors associated with changes in mortality of females with NSCLC

Factor	Univariate analysis (N = 124)		Multivariate analysis (N = 107)	
	Hazard ratio (95% confidence limits)	P	Hazard ratio (95% confidence limits)	P
PTHrP negative	1		1	
PTHrP positive	0.51 (0.34-0.77)	0.002	0.62 (0.4-0.99)	0.047
Stage I	1		1	
Stage II	2.64 (0.94-7.36)	0.016	3.94 (1.33-11.73)	0.012
Stage III	3.06 (1.73-5.43)	<0.001	4.50 (2.43-8.34)	<0.001
Stage IV	6.06 (3.00-12.23)	<0.001	14.26 (6.47-31.44)	<0.001
Adenocarcinoma	1		1	
Large cell	2.00 (1.01-3.98)	0.049	3.72 (1.40-9.86)	<0.001
Squamous	1.74 (1.14-2.66)	0.010	2.00 (1.21-3.29)	<0.001
Age	1.04 (1.02-1.06)	<0.001	1.07 (1.04-1.10)	<0.001

NOTE: Ps are calculated from the likelihood ratio test. Age hazard is expressed per year.

never smokers or had a smoking history (Fig. 3). For the smokers, median survival times were 46 months for PTHrP-positive patients and 14 months for PTHrP-negative patients (P = 0.037). Median survival for women who had never smoked and had PTHrP-positive tumors was not reached by 200 months after diagnosis (range, 50-200 months; n = 11) but was only 20 months when tumors did not express PTHrP (P = 0.004). Survival curves for male smokers in our patient population were not affected appreciably by PTHrP status.

Because the distribution of PTHrP in females varied significantly with a number of factors that are potential confounders of the relationship with survival, we also analyzed survival in more homogeneous subgroups of patients that shared either the same hospital source, histology, stage, smoking history, or age range. In each case, median survival was greater for PTHrP-positive females than for the corresponding PTHrP-negative patients in each subgroup (Table 4). In most cases, the difference by log-rank test was statistically significant. In females ages <67 years, P = 0.066, not quite meeting the level for significance. We split this group in half by age at its median of 56 years and examined the younger set to determine whether the prognostic relationship with PTHrP might be restricted to older patients. However, in female cancer patients ages <56 years, median survival was 71 months for PTHrP-positive individuals versus 20 months for those whose tumors lacked the protein (P = 0.024). In a multivariate Cox regression analysis of the UCSD female group (n = 80), stage, histology, and age were significant predictors of survival (P < 0.05), but PTHrP was not (P = 0.19).

An evaluation of the factors that varied between male and female patients could provide an explanation for the sex-dependent relationship between PTHrP. The only prognostic indicators that differed by sex were smoking history and stage. A history of never having smoked was present in 15.4%

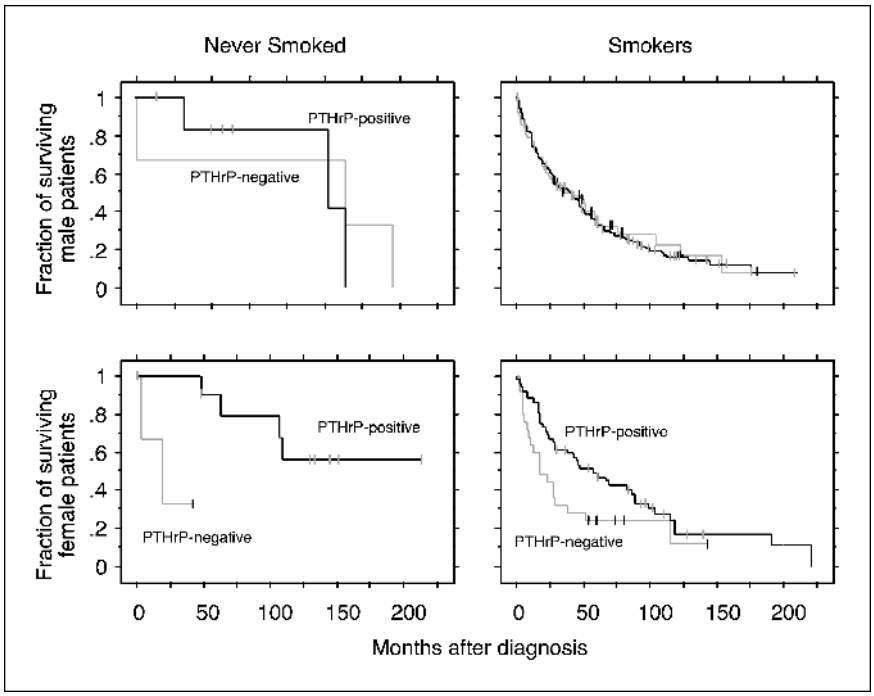

Fig. 3. Survival curves for nonsmokers versus smokers. Smoking history and PTHrP were both associated with differences in survival in univariate analyses. Therefore, we examined individual survival curves for female and male nonsmokers and smokers. The number of males who never smoked was too small for a meaningful analysis (*top left*). In male smokers (*top right*), the survival curves were not appreciably affected by lung carcinoma PTHrP expression (*gray*, PTHrP negative; *black*, PTHrP positive). In contrast, longer survival times were observed for female patients with PTHrP-positive NSCLC than for patients with PTHrP-negative tumors, regardless of whether the patients had never smoked or had a history of smoking. Log-rank tests were significant at P = 0.037 for female smokers and P = 0.004 for female patients who never smoked. The data include three nonsmoking females who were PTHrP negative (two events) and 11 who were PTHrP positive (four events). The plot for female smokers represents 25 PTHrP-negative patients (20 events) and 52 PTHrP-positive patients (40 events). Tick marks mark the points at which surviving patients were censored from the analysis.

Table 4. Cox survival analysis for homogeneous groups of female patients

	Survival (mo), median quartiles	*n*	Log-rank *P*	Survival (mo), median quartiles	*n*	Log-rank *P*
	UCSD patients			Cytomyx patients		
PTHrP positive	63 (20, 126)	66	<0.001	55 (31, 80)	11	0.213
PTHrP negative	14 (6, 41)	30		24 (20, 80)	16	
	Adenocarcinomas			Squamous carcinomas		
PTHrP positive	66 (31, 126)	46	0.045	46 (17, 95)	26	0.025
PTHrP negative	23 (16, 92)	16		16 (6, 30)	21	
	Stage I + II			Stage III + IV		
PTHrP positive	69 (34, 116)	48	0.030	25 (14, 95)	29	0.001
PTHrP negative	27 (20, 97)	27		8 (4, 20)	19	
	Smokers			Nonsmokers		
PTHrP positive	46 (14, 92)	52	0.037	NR (107, NR)	11	0.004
PTHrP negative	14 (6, 41)	25		20 (3, NR)	8	
	Age ≥ 67 y			Age < 67 y		
PTHrP positive	34 (19, 71)	32	0.005	71 (34, 176)	45	0.066
PTHrP negative	16 (7, 23)	28		41 (14, 94)	18	
	Age < 56 y					
PTHrP positive	71 (38, 176)	23	0.024			
PTHrP negative	20 (14, 92)	8				

NOTE: Log-rank *P* is for the comparison between PTHrP-negative and PTHrP-positive patients. There were only eight female patients with large cell carcinoma.
Abbreviation: NR, not reached.

(14 of 91 patients) of the females compared with 4.5% (10 of 204) of the males ($P = 0.004$). Approximately 70% (75 of 107) of the females had stage I disease versus 60% (150 of 250) of the males ($P = 0.004$). These differences are small and do not give great cause to suspect that the sex-dependent PTHrP effect depends on stage or smoking. Histology and age did not differ significantly between the groups.

Intracellular PTHrP immunoreactivity could be found in both the nucleus and cytoplasm (Fig. 4) but not in every patient. About half of the tumors had a solely cytoplasmic distribution, regardless of sex (data not shown). Patient survival did not differ as a function of whether PTHrP was restricted to the cytoplasm or also appeared in the nucleus for either males or females.

Discussion

In a previous study, we obtained evidence that PTHrP could have a role in regulating progression of lung cancer. We introduced PTHrP-expressing orthotopic lung carcinomas into athymic mice and treated them with neutralizing antibodies to PTHrP (1). Control animals received irrelevant isotype control antibody. Antibody treatment significantly reduced serum and tumor PTHrP levels. After 4 weeks of treatment, the animals treated with the PTHrP antibody had greater tumor burden than the control animals. These data suggested that endogenous PTHrP restrained the growth of orthotopic lung carcinomas, and that reducing levels of the hormone contributed to a more rapid increase in tumor mass. We also showed that PTHrP inhibited proliferation of the same lung cancer cells when grown in culture, providing a plausible cellular mechanism, whereby the hormone might slow tumor growth. Human lung carcinomas frequently express PTHrP, in roughly half to three quarters of non–small cell tumors (26–29), and effects on lung carcinoma growth could conceivably alter patient survival. Thus, we conducted the study described in this article to investigate whether PTHrP was a prognostic factor in lung cancer.

In our patient population, expression of PTHrP by NSCLC tumors was associated with increased survival time in women but not men. The difference in median survival time between the PTHrP-positive and PTHrP-negative female groups was over

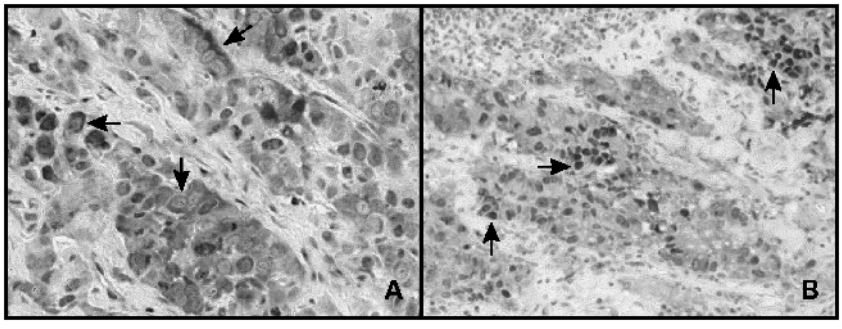

Fig. 4. Intracellular distribution of PTHrP immunoreactivity in lung carcinomas. *A,* large cell carcinoma (a portion of Fig. 1E magnified ×2) with predominantly cytoplasmic PTHrP reaction product outlining the nuclei. *B,* adenocarcinoma with predominantly nuclear PTHrP staining. Arrows mark representative cells.

3 years. Associations between PTHrP and prolongation of survival have also been observed in studies of pancreatic cancer and breast cancer. Renal carcinoma patients with strong tumor PTHrP staining using the same COOH-terminal PTHrP antibody we used had 87% of 5-year disease-free survival compared with 50% for patients with minimal staining (30). In breast carcinomas, PTHrP 1-14 immunoreactivity was associated with a smaller yet statistically significant increase in 5-year survival rate, 87% (95% confidence interval, 82-91%; $n = 265$) compared with 73% (63-81%; $n = 102$) for patients with PTHrP-positive and PTHrP-negative tumors, respectively. The known biological effects of PTHrP could reasonably account for these findings. For example, PTHrP has been reported to inhibit growth of some lines of breast carcinoma cells (2), as it did for the lung cancer cells in our previous experiment (1). In addition, PTHrP reduces angiogenesis in some cancer models (13), a factor that could also reduce carcinoma progression.

Two previous publications have investigated PTHrP and lung cancer progression in patients. One publication was a case report of a patient with small cell lung carcinoma. Malignant pleural cells harvested at two separate times produced increased levels of PTHrP 1-34 at the later time when the cancer had become more poorly differentiated (31). We did not perform serial measurements nor did we measure PTHrP production by harvested cells. We can state that tumor PTHrP 109-141 immunoreactivity was not linked to stage for the patients with non–small cell cancer in our study. Another study investigated survival of NSCLC patients with hypercalcemia (32). Seven patients with high serum levels of PTHrP, measured with a COOH-terminal PTHrP assay, had a median survival time of 1.4 months, whereas 16 patients with lower serum PTHrP survived a median time of 5.4 months. This small investigation differs from ours in suggesting a negative prognostic relationship for PTHrP, but it also studied a different patient population, one restricted to hypercalcemic patients and with shorter survival times, on average, than our study. Comparison to our results is problematic because of differences in the type of lung cancer, patient population, assay method, and design.

The clinical studies of PTHrP expression described above used antibodies specific for NH_2-terminal or COOH-terminal PTHrP regions, an important point because different portion of the PTHrP molecule are biologically active and could vary in significance for a disease process. NH_2-terminal (PTHrP 1-34 or 1-36), midmolecule (PTHrP 38-94 or 38-102), and COOH-terminal regions (PTHrP 109-141) have effects in different systems, although a receptor has only been identified at this time for the NH_2-terminal region, PTH1R (33). Peptides in the COOH-terminal PTHrP domain are active against osteoclasts (34) but direct effects on cancer cells have not been described. The studies in which PTHrP had a positive prognostic role in cancer used an antibody to PTHrP 1-14 for breast cancer, one directed against PTHrP 126-136 in renal cell carcinoma and our study (1, 18, 30). Furthermore, the small cell lung cancer case report and the NSCLC case series in which PTHrP apparently associated with more aggressive malignancies used NH_2-terminal and COOH-terminal PTHrP antibodies, respectively (31, 32). Thus, study results do not align based on PTHrP epitope.

Further work will be needed to determine whether the association between PTHrP and survival can be generalized beyond this study to lung cancer patients in general. The study included multiple subgroups made up by different institutions, histologies, stages, and other factors. The VASDHS patients were all male, consistent with the prevailing sex distribution in that hospital population. The UCSD and Cytomyx patients had a higher frequency of adenocarcinomas than the VA group, which was weighed more toward squamous carcinomas. This finding might be explained by the higher percentage of female patients, who are more likely to have adenocarcinomas than male patients (35). The Cytomyx patients were more likely to have stage I cancers and cancers that were PTHrP-negative than the other groups. Finally, the Cytomyx group differed in that the analyses were done on a tissue microarray, whereas the VASDHS and UCSD patient material consisted of sections cut from individual blocks. The inhomogeneities among the groups sets up the potential that an unrecognized confounding factor might produce an apparent association between PTHrP and survival.

The potential covariates that could confound our analysis include histology, age, and smoking history. In females, the presence of PTHrP varied with each factor and all were associated with differences in NSCLC patient life span. PTHrP expression also varied with patient source, being less frequent in the Cytomyx patients, but patient source was not a univariate predictor of survival. We did additional statistical inquiries to explore the strength of the relationship between PTHrP and survival. In a multivariate analysis, PTHrP remained an independent predictor of increased longevity for women with NSCLC even after controlling for the effects of histology and age, as well as stage. Furthermore, analyses of more homogenous subgroups, based on a single hospital site, histology, stage grouping, smoking history, or age range, showed that PTHrP-positive females had higher median survival times than their PTHrP-negative counterparts within the group. In most cases, the differences within the more homogeneous groups based on PTHrP were significant. Thus, we could not identify covariates that might confound the relationship between PTHrP and survival. Still, reservations remain due to the small size of the subgroups and the differences among them. The results generate a cogent hypothesis regarding how PTHrP could affect survival and a larger study with complete data should be undertaken to validate the conclusions.

Our study, one of the largest to measure PTHrP in lung cancer patients, verified previous reports that PTHrP is expressed in the majority of human non–small cell lung tumors (26–29). PTHrP has been reported to be a product of alveolar type II cells in normal adult lung in rat and rabbit, but we found minimal expression in normal lung specimens from our patients. Interestingly, PTHrP expression was increased in normal lung obtained from patients with lung carcinoma compared with samples from patients without cancer. This observation suggests that the lung tumor could be interacting with adjacent lung or that benign tissue could take up tumor-derived PTHrP.

Sex-based effects of PTHrP on survival have not been reported previously in cancer to our knowledge but are reasonable given the complicated interactions between hormones, sex-related factors, and cancer. Interestingly, our previous mouse orthotopic lung carcinoma study used female mice (1). Thus, both studies found positive results in PTHrP seemed to exert favorable effects for females with lung cancer. The animal study did not include males; thus, we cannot say whether the effects of PTHrP on lung cancer progression in mice depended on sex. Sex-specific PTHrP effects are recognized in other settings; thus, it is not surprising to find these differences in lung cancer. For example, PTHrP

augments coronary blood flow to a greater extent in female rats than in male rats (36). In addition, PTHrP stimulates nipple development in fetal mice but only in females (37). A sex-based distinction in PTHrP effects in lung cancer could stem from a number of stimuli, including environmental differences or behavioral features, genetic influences, and hormonal effects. Each of these factors is thought to contribute to sex differences in lung cancer risk and survival and could modulate the PTHrP response, as well (38).

We evaluated whether the distribution of PTHrP between cytoplasm and nucleus was important for lung cancer survival because the molecule exerts effects in a number of cancer cells after localizing to the nucleus. The intracrine (nuclear) actions of PTHrP may modify growth rates or alter sensitivity to apoptosis (5–7), events that could conceivably influence carcinoma growth and affect outcome. Survivin, a protein that inhibits apoptosis, serves as an example of how intracellular location may vary among patients and alter lung cancer outcome. Nuclear survivin increases relative risk of death by almost 4-fold whereas cytoplasmic protein confers minimal change in risk (39). In our study, however, survival of female patients was increased regardless of whether PTHrP migrated to the nucleus or remained solely cytoplasmic. Thus, we have no reason to believe that nuclear localization of PTHrP is important for the survival benefit.

This investigation indicates that immunohistochemical staining for PTHrP in NSCLC could be valuable in gauging the prognosis of female patients. Combined with previous animal work and knowledge about how PTHrP regulates cancer cells, it also suggests that PTHrP could play a direct role in modifying longevity of women with NSCLC. Additional research will be necessary to verify the result and to explore mechanisms.

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