Featured Article

Serum Levels of Insulin Growth Factor (IGF-I) and IGF-Binding Protein Predict Risk of Second Primary Tumors in Patients with Head and Neck Cancer

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ABSTRACT

Purpose: **Second primary tumors (SPTs) are a hallmark of head and neck squamous cell carcinomas (HNSCCs). Serum levels of insulin growth factors (IGFs) and their binding proteins (IGFBPs) have been associated with subsequent development of several epithelial cancers in prospective studies.**

Experimental Design: **To examine the role of IGFs in SPT development, we conducted a nested case–control study within a randomized, placebo-controlled chemoprevention trial in patients with early-stage HNSCC. We compared prediagnostic serum IGF-I and IGFBP-3 levels in 80 patients who subsequently developed SPTs and 173 controls (patients without SPTs) matched to the cases on age (±5 years), sex, ethnicity, year of randomization, and length of follow-up.**

Results: **The cases exhibited significantly higher levels of IGF-I and IGFBP-3 than did the controls ($P = 0.001$ and 0.019, respectively). Elevated IGF-I levels were associated with a 3.66-fold significantly increased risk of SPT. Lower and higher IGFBP-3 levels were associated with a 2.22- and 7.12-fold significant increased risk, respectively. The median SPT-free time was significantly shorter in patients with higher IGF-I levels than in patients with lower IGF-I levels ($P < 0.0001$). A similar trend was observed for IGFBP-3 ($P = 0.002$). Moreover, in the Cox proportional hazards model, higher IGF-I levels were significantly associated with increased risk of SPT with a hazard ratio of 2.78. Patients with the lower and higher IGFBP-3 levels also exhibited significantly increased risks with hazard ratios of 1.65 and 2.17, respectively.**

Conclusions: **This is the first study demonstrating that higher IGF-I levels, and lower and higher IGFBP-3 levels are risk factors for SPT development. Thus, measuring serum IGF-I and IGFBP-3 levels may be useful markers in assessing the risk of second tumors in patients successfully treated for their index cancer.**

INTRODUCTION

Head and neck cancers accounted for 2.8% of all incident cancers and 2.0% of fatal cancers in the United States in 2003 (1). These statistics represent 37,200 new cases annually and 11,000 deaths. A major cause of failure in early-stage patients is the development of second primary tumors (SPTs; Refs. 2–6). Patients with an index cancer of the upper aerodigestive tract incur a 4–7% annual risk of developing a potentially fatal SPT (7–10), and this risk of developing a SPT does not decrease over time (9, 11). As diagnostic and therapeutic procedures continue to improve, it is likely that the problem of SPTs will assume an even greater relevance for treatment and prognosis.

The etiological role of tobacco and alcohol exposures in upper aerodigestive cancers is unquestioned. Analogous to lung cancers, upper aerodigestive tract cancers are considered prototypes of environmentally induced diseases. Blot *et al.* (12) attribute approximately three-fourths of all oral and pharyngeal cancers in the United States to tobacco smoking and alcohol consumption. However, only a fraction of exposed individuals will develop neoplastic lesions; thus, the concept of genetic susceptibility to carcinogenic exposures must be factored into the risk assessment analysis, both for index cancers and for the evaluation of risk for multiple cancers.

Recent studies have implicated insulin-like growth factors (IGFs), specifically IGF-I, and its binding protein 3 (IGFBP-3) in cancer development. IGF-I plays an essential role in regulating cell proliferation and differentiation. These actions are executed via the IGF receptor I (IGF-IR), which allows IGF-I to exert its mitogenic effect on both normal and cancer cells (13–14). IGF-IR possesses tyrosine kinase activity inducing both ras and phosphatidylinositol 3′-kinase-related signal transduction pathways. In addition, IGF-I is also involved in other aspects of cancer development and progression, such as angiogenesis and inflammation (15–16). Furthermore, IGFs can suppress cellular apoptotic pathways and facilitate cell growth (17–19). The functions of IGF-I are partly regulated by IGFBP-3; more than 90% of circulating IGF-I is complexed with IGFBP-3. IGFBP-3 normally inhibits the mitogenic action of IGF-I by preventing it from binding to its receptor; however, under certain circumstances, this binding can enhance the activity of IGF-I by protecting it from degradation (20), although the exact mechanism by which this occurs is unclear. IGFBP-3 can also act independently by interacting with a number of cellular

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proteins. Overexpression of IGFBP-3 has also been related to apoptosis (21–22).

There is considerable individual variability in circulating levels of IGF-I and IGFBP-3. Recent prospective and retrospective studies have demonstrated that elevated serum IGF-I levels are associated with increased risk of a variety of epithelial cancers (23–34). However, the results for IGFBP-3 are more controversial. Some studies show that elevated serum levels of IGFBP-3 are associated with either increased or decreased cancer risk (24–32), whereas other studies do not detect an association between serum levels of IGFBP-3 and cancer risk (23, 35). This inconsistency might be because of the dual role of IGFBP-3. To determine whether IGF-I and IGFBP-3 could serve as serum markers for predicting or diagnosing SPTs, we performed a nested case–control study within a chemoprevention trial that was designed to decrease the risk of secondary cancer, to compare prediagnostic serum IGF-1 and IGFBP-3 levels in patients with and without SPTs.

MATERIALS AND METHODS

Study Population. We designed this nested case–control study within the Retinoid Head and Neck Second Primary (HNSP) Trial that began in November 1991 and closed to new patient accrual in September 1999. A detailed description of this study has been published previously (35, 36). Briefly, this placebo-controlled, double-blinded study evaluated the efficacy of low-dose 13-*cis*-retinoic acid (13 cRA) in the prevention of SPTs. Patients with histologically confirmed stage I or II squamous cell carcinoma of the larynx, oral cavity, or pharynx (as defined by the American Joint Committee Staging criteria), recruited within 36 months of diagnosis, were enrolled from the Radiation Therapy Oncology Group, the University of Texas M.-D. Anderson Cancer Center, the Clinical Community Oncology Group, and the Southwest Oncology Group. In addition, the patients had been successfully treated with surgery, radiotherapy, or both, and were cancer free for at least 16 weeks at the time of recruitment. Excluded were patients with concurrent malignancies other than localized nonmelanoma skin cancer, patients with multiple head and neck primary tumors, and patients with a prior history of cancer in sites other than the head and neck region. Patients were assigned with equal probability to either the 13 cRA treatment or the placebo arm. The stage (I or II), primary tumor site (larynx, oral cavity, or pharynx), and smoking status (never/former, recent/current smoker) were used as stratifying factors to allocate treatment before randomization. Follow-up information on SPTs and primary tumor recurrence was collected every 3, 4, or 6 months from the commencement of the trial. An SPT was defined using the Warren and Gates criteria (37). Specifically, a tumor was considered to be an SPT, if it was (*a*) a new cancer of a different histology from the initial primary tumor; (*b*) or the same histology but occurring more than 3 years after therapy of the primary tumor; or (*c*) more than 2 cm from the primary tumor, separated by clinically normal epithelium. This research was approved by all of the relevant review boards and in accordance with an assurance filed with, and approved by, the United States Department of Health and Human Services. Consent forms were obtained from all participants.

A total of 1384 patients were registered, of whom 1191 patients were randomized into the study. As of September 1, 2002, 238 patients were diagnosed with SPTs. Serum that had been drawn at enrollment (before development of the SPT) was available for 80 cases. The median duration between blood drawn and SPT development was ~32 months (range, 6–69 months). Controls (patients without SPTs) were randomly chosen from the entire pool of patients in the trial who did not develop SPTs and were frequency-matched to the cases on age (± 5 years), sex, ethnicity, year of randomization, and length of follow-up in a 1:2 ratio of cases:controls. Because the majority of the participants were Caucasians, our analyses were limited to this group. The final analysis consisted of 80 SPT cases and 173 controls.

Data Collection. Before randomization, participants were given a structured questionnaire that elicited information on sociodemographic factors, clinical information, tobacco exposure, and alcohol consumption. After the interview, 30 ml of blood was collected in standard clot tubes (red-top tube). The serum was then separated by centrifugation and placed in labeled polypropylene tubes, wrapped in aluminum foil (protected from light), and stored frozen (−70°C) until shipped for assay. The samples were shipped by overnight delivery with a cold pack in a Styrofoam shipping container and stored at −80°C until analysis.

Measurements of IGF-I and IGFBP-3. Two commercially available ELISA kits from Diagnostic Systems Laboratories (Webster, TX) were used to determine the serum levels of total IGF-I (DSL-10-5600) and IGFBP-3 (DSL-10-6600). Cross-reaction of the antibodies with other members of the IGF family is not detected at physiological concentrations, according to the manufacturer. The assays were performed following the instructions of the manufacturer (DSL) and laboratory personnel were blinded to the case or control status of the samples. We separated IGF-I from their binding proteins by mixing serum specimens with acid-ethanol extraction buffer, supplied with the kits, and measured the serum levels of total IGF-I. For IGFBP-3, the specimens were diluted 100-fold in an assay buffer before conducting the ELISA test. All specimens were assayed twice for IGF-I and IGFBP-3, and the average of the two measurements was used in the data analysis.

Statistical Analysis. Spearman correlation coefficients were used to examine the correlation among IGF-I, IGFBP-3, IGF-I/IGFBP-3, and age. The Kruskal-Wallis test was used to test the correlation among IGF-I, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio and sex, cigarette smoking status, and treatment regimen. The distributions of the variables studied between the cases and controls were compared using the χ^2 test for categorical variables (sex and smoking status) and the Kruskal-Wallis test for continuous variables (age, IGF-I, IGFBP-3, and IGF-I/IGFBP-3). Because the distributions of IGF-I and IGFBP-3 in the population were positively skewed, the levels of IGF-I and IGFBP-3 were categorized using recursive-partitioning procedures (*i.e.*, RPART in S-plus). This recursive partitioning methodology does not depend on any underlying distributional assumptions and allows for nonlinear relations between predictive factors and outcomes. The cutpoints obtained correspond to the lowest cross-validation error rates, which are preferred to the usual straightforward method of using tertiles or quartiles as cutpoints.

Table 1 Levels of IGF-I,[a] IGFBP-3 and IGF-I/IGFBP-3 molar ratio in SPT patients and controls
All P values are two-sided, and associations are considered statistically significant at $P < 0.05$.

Variables	Controls ($n = 173$)	SPTs	
		Total ($n = 80$)	Smoking-related[b] ($n = 58$)
Age (yr)			
Median (range)	66 (39–85)	66.5 (38–82)	66.5 (38–81)
P value[c]		0.493	0.866
Gender			
Male	34 (19.7%)	16 (20.0%)	12 (20.7%)
Female	139 (80.4%)	64 (80.0%)	46 (79.3%)
P value[d]		0.949	0.864
Smoking status			
Never	24 (13.9%)	6 (7.5%)	4 (6.9%)
Former	100 (57.8%)	37 (46.3%)	26 (44.8%)
Current	49 (28.3%)	37 (46.3%)	28 (48.3%)
P value[d]		0.015	0.016
Treatment regimen			
Placebo	88 (50.9%)	46 (57.5%)	35 (60.3%)
13 cRA	85 (49.1%)	34 (42.5%)	23 (39.7%)
P value[d]		0.326	0.211
IGF-I ng/ml			
Median (range)	93.61 (17.19–352.33)	134.85 (20.14–422.27)	137.97 (20.14–422.27)
P value[c]		0.001	0.002
IGFBP-3 ng/ml			
Median (range)	2405.8 (256.8–6421.6)	2969.6 (708.6–5538.6)	2999.2 (708.6–5538.6)
P value[c]		0.019	0.004
IGF-I/IGFBP-3 molar ratio			
Median (range)	0.15 (0.02–0.83)	0.17 (0.02–1.09)	0.16 (0.02–1.09)
P Value[c]		0.044	0.224

[a] IGF-I, insulin-like growth factor-I; IGFBP-3, IGF-binding protein-3; SPT, second primary tumors; 13 cRA, 13-*cis*-retinoic acid.
[b] Smoking-related SPTs include larynx, oral cavity, pharynx, lung, and bladder tumors.
[c] Kruskal-Wallis test was used to calculate the *P* value.
[d] χ^2 test was used to calculate the *P* value.

In addition to the default tree generated by the recursive partitioning algorithm, we examined alternative initial splits using systematic inspection and assessed the sensitivity of the chosen splits via bootstrap simulations. To assess the strength of the association between SPT risk and the growth factors, we calculated odds ratios (OR) and their corresponding 95% confidence intervals (CI) using both univariate and multivariate unconditional logistic regression models. In addition, we also evaluated the strength of associations between smoking-related SPTs and the growth factors. In the multivariate analysis, we adjusted for possible confounders such as sex, age, cigarette smoking status (never, former, or current) and treatment regimen (placebo, 13 cRA). Because sex and age were not statistically significant in the model, these two variables were dropped out in the final model. Former smokers were individuals who had successfully stopped smoking at least 1 year before enrolling into the study. Never smokers were individuals who had smoked less than 100 cigarettes during their lifetime. The interaction between IGF-I and IGFBP-3 was also examined in the logistic regression model by creating a term that is the product of the two variables. The Kaplan-Meier estimate was computed to estimate the probability of SPT-free survival. The Cox proportional hazards model was applied to analyze the effect of IGF-I, IGFBP-3, and these factors combined in association with the development of SPTs. All *P* values were two-sided. Associations were considered statistically significant at $P < 0.05$.

RESULTS

The nested case–control sample contained 173 patients without SPTs (controls) and 80 patients with any SPT (cases) of whom 58 patients had smoking-related SPTs (8 larynx, 13 oral cavity, 3 pharynx, 32 lung, and 2 bladder) and 22 patients had nonsmoking-related SPTs (9 prostate, 2 colon, 2 kidney, 2 liver,

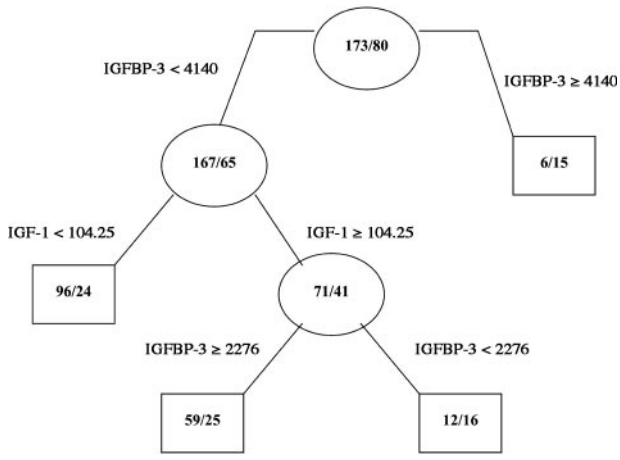

Fig. 1 Recursive-partitioning procedure to predict second primary tumor risk (number of controls/number of cases).

Table 2 Risk estimates [*n* (%)] for all SPTs[a] relative to IGF-I, IGFBP-3 levels, and smoking status in logistic regression analysis

	No SPT ($n = 173$)	SPT ($n = 80$)	Univariate OR (95% CI)	Multivariate OR (95% CI)[b]
Smoking status				
Never	24 (13.9)	6 (7.5)	1.00	1.00
Former	100 (57.8)	37 (46.3)	1.48 (0.56–3.91)	1.32 (0.47–3.74)
Current	49 (28.3)	37 (46.3)	3.02 (1.12–8.14)	2.54 (0.88–7.38)
Treatment regimen				
Placebo	88 (50.9)	46 (57.5)	1.00	1.00
13 cRA	85 (49.1)	34 (42.5)	0.77 (0.45–1.31)	0.72 (0.40–1.32)
IGF-I (ng/ml)				
< 104.25	98 (57.7)	25 (31.6)	1.00	1.00
≥ 104.25	72 (42.3)	54 (68.4)	2.94 (1.67–5.16)	3.66 (1.86–7.19)
IGFBP-3 (ng/ml)				
$\geq 2276, < 4140$	91 (53.2)	32 (40.5)	1.00	1.00
< 2276	74 (43.3)	32 (40.5)	1.23 (0.69–2.19)	2.22 (1.11–4.45)
≥ 4140	6 (3.5)	15 (19.0)	7.11 (2.54–19.89)	7.12 (2.32–21.80)

[a] SPT, second primary tumor; IGF-I, insulin-like growth factor-I; IGFBP-3, IGF binding protein 3; OR, odds ratio; CI, confidence interval; 13 cRA, 13-cis-retinoic acid.

[b] Multivariate logistic regression model was built to assess the predictive effects of IGF-I and IGFBP-3 for SPT risk. The model simultaneously includes IGF-I, IGFBP-3, smoking status at registration, and treatment regimen.

1 bone marrow, 1 breast, 1 small intestine, and 4 unknown). By design, the cases and controls were well matched in terms of age and gender (Table 1). The cases were significantly more likely than controls to be current smokers (46.3 *versus* 28.3%), and less likely to be never smokers (7.5 *versus* 13.9%; $P = 0.015$). Overall, 46 (34%) of the patients in the placebo arm presented with SPTs compared with 34 (28.6%) of the patients treated with 13 cRA ($P = 0.33$). Serum levels of IGF-I were significantly correlated with serum levels of IGFBP-3 ($r = 0.47$; $P < 0.001$) and the molar ratio of IGF-I/IGFBP-3 ($r = 0.69$; $P < 0.001$; data not shown). Serum levels of IGFBP-3 were significantly inversely correlated with age ($r = -0.19$; $P = 0.002$) and the molar ratio of IGF-I/IGFBP-3 ($r = -0.25$; $P < 0.001$). Men had higher serum levels of IGF-I ($P = 0.026$) and molar ratios of IGF-I/IGFBP-3 ($P < 0.001$) than women. However, there was no correlation between IGF-I, or IGFBP-3, and smoking status (data not shown). In the control patients, comparing the placebo arm group with the 13 cRA treatment group, serum levels of IGF-I, IGFBP-3, and the molar ratios of IGF-I/IGFBP-3 were not significantly different between the two treatment groups ($P = 0.535$, $P = 0.167$, and $P = 0.192$, respectively). Cases treated with 13 cRA had marginally significantly lower median levels of IGF-I (119.04 *versus* 151.75 ng/ml; $P = 0.074$) and significantly lower median levels of IGFBP-3 (2527.64 *versus* 3183.14 ng/ml; $P = 0.027$); however, the molar ratios of IGF-I/IGFBP-3 were not significantly different between the two treatment groups ($P = 0.713$). The median levels of IGF-I and IGFBP-3 were significantly higher in the cases than in the controls (134.85 *versus* 93.61 ng/ml, $P = 0.001$; 2969.6 *versus* 2405.8 ng/ml, $P = 0.019$, respectively; Table 1). Additionally, there was a statistically significant difference in the median molar ratio of IGF-I/IGFBP-3 between the cases and the controls (0.17 *versus* 0.15, $P = 0.044$). Similar trends were observed when comparing patients with smoking-related SPTs and controls except for difference in the median molar ratio of IGF-I/IGFBP-3.

Table 3 Risk estimates [*n* (%)] for smoking related-SPT[a] risk in relation to IGF-I, IGFBP-3, and smoking status in logistic regression analysis

	No SPT ($n = 173$)	SPT[b] ($n = 58$)	Univariate OR (95% CI)	Multivariate OR (95% CI)[c]
Smoking status				
Never	24 (13.9)	4 (6.9)	1.00	1.00
Former	100 (57.8)	26 (44.8)	1.56 (0.50–4.89)	1.48 (0.43–5.14)
Current	49 (28.3)	28 (48.3)	3.43 (1.08–10.89)	3.22 (0.91–11.38)
Treatment regimen				
Placebo	88 (50.9)	35 (60.3)	1.00	1.00
13 cRA	85 (49.1)	23 (39.7)	0.68 (0.37–1.25)	0.71 (0.37–1.40)
IGF-I (ng/ml)				
< 104.25	98 (57.7)	19 (32.8)	1.00	1.00
≥ 104.25	72 (42.3)	39 (67.2)	2.79 (1.49–5.23)	2.90 (1.37–6.13)
IGFBP-3 (ng/ml)				
$\geq 2276, < 4140$	91 (53.2)	25 (43.1)	1.00	1.00
< 2276	74 (43.3)	20 (34.5)	0.98 (0.51–1.91)	1.61 (0.74–3.51)
≥ 4140	6 (3.5)	13 (22.4)	7.88 (2.72–22.84)	8.27 (2.60–26.28)

[a] SPT, second primary tumor; IGF-I, insulin-like growth factor-I; IGFBP-3, IGF binding protein 3; OR, odds ratio; CI, confidence interval; 13 cRA, 13-cis-retinoic acid.

[b] Smoking-related SPTs include larynx, oral cavity, pharynx, lung, and bladder tumors.

[c] Multivariate logistic regression model was built to assess the predictive effects of IGF-I and IGFBP-3 for smoking-related SPT risk. The model simultaneously includes IGF-I, IGFBP-3, smoking status at registration, and treatment regimen.

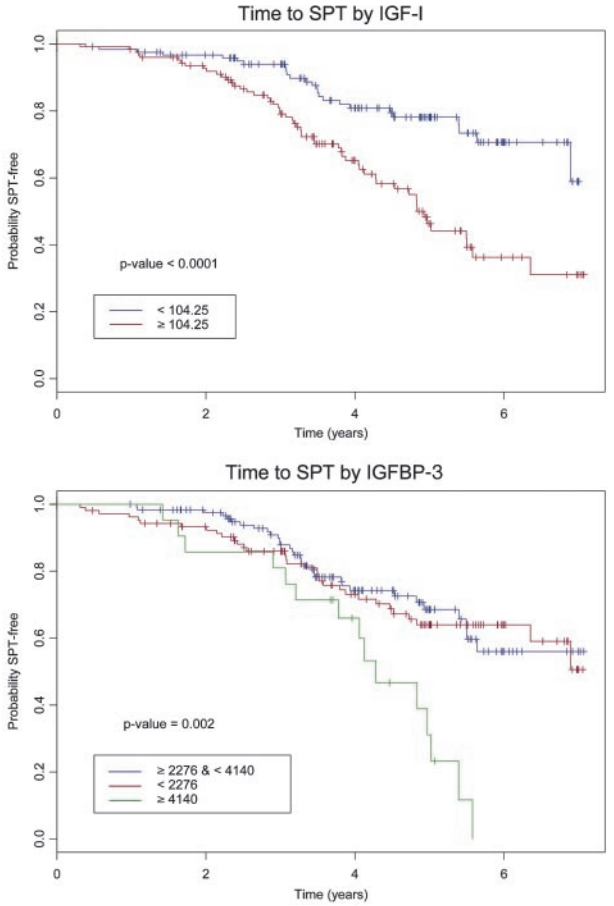

Fig. 2 Second primary tumor (SPT)-free survival curves: survival stratified by the insulin-like growth factor-I (IGF-I) and IGF binding protein 3 (IGFBP-3).

Recursive-partitioning procedures were used to build a multivariate logistic model to predict the risk of SPT. In addition to IGF-I and IGFBP-3, age, gender, smoking status, and treatment regimen were included in the multivariate logistic model. SPT response was explained best by IGF-I and IGFBP-3 in which a single cutpoint, 104.25 ng/ml, was defined for IGF-I and two cutpoints, 2276 and 4140 ng/ml, were defined for IGFBP-3 (Fig. 1). To determine whether IGF-I and IGFBP-3 serum levels were associated with SPT risk, we conducted both univariate and multivariate unconditional logistic regression analyses (Table 2). Current smoking at time of randomization was associated with a 2.5-fold increased risk of SPT (OR, 2.54; 95% CI, 0.88–7.38). Treatment with 13 cRA was associated with an almost 30% reduction in risk, but this did not achieve statistical significance. After adjustment for smoking status and treatment regimen in the multivariate model, elevated serum levels of IGF-I were positively associated with the risk of SPTs with an OR of 3.66 (95% CI, 1.86–7.19), using the cutoff point of 104.25 ng/ml as defined by the recursive-partitioning procedure. Using patients with a midlevel of IGFBP-3 as the reference group (≥2276 and <4140 ng/ml), patients with either lower (<2276 ng/ml) or higher levels of IGFBP-3 (≥4140 ng/ml) exhibited increased risks of developing SPTs, with ORs of 2.22 (95% CI, 1.11–4.45) and 7.12 (95% CI, 2.32–21.80), respectively. Similar trends were observed specifically for smoking-related SPTs (Table 3).

Kaplan-Meier cumulative incidence curves were used to estimate time to SPT (Fig. 2). Patients with higher levels of IGF-I (≥104.25 ng/ml) exhibited a shorter median SPT-free survival time (4.93 months) compared with patients with lower levels of IGF-I (<104.25 ng/ml; median not attained; $P < 0.0001$). Patients with the highest levels of IGFBP-3 (≥4140 ng/ml) had the shortest SPT-free survival time (4.28 months). The median SPT-free survival time was not attained for individuals with lower and middle levels of IGFBP-3 ($P = 0.002$). Once again, similar trends were observed for smoking-related SPT-free survival curves (Fig. 3).

In the Cox proportional hazard model, IGF-I and IGFBP-3 levels both were significant positive predictors of SPT development after adjusting for smoking status at randomization and treatment regimen (Table 4). For IGF-I, the hazard ratio was 2.78 (95% CI, 1.62–4.77). Patients with lowest and highest levels of IGFBP-3 had hazard ratios of 1.65 (95% CI, 0.96–2.84) and 2.17 (95% CI, 1.14–4.13), respectively. Current smokers had an almost 2-fold increased

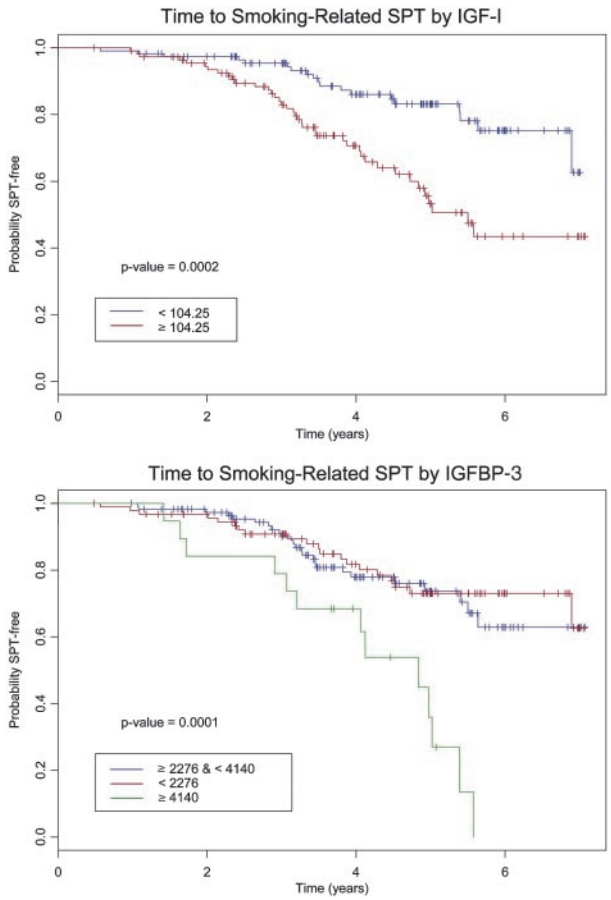

Fig. 3 Smoking-related second primary tumor (SPT)-free survival curves: survival stratified by the insulin-like growth factor-I (IGF-I) and IGF binding protein 3 (IGFBP-3).

Table 4 Cox proportional hazards model of time to development of SPT[a] (all SPTs)

	No SPT (n = 173)	SPT (n = 80)	Univariate HR (95% CI)	Multivariate HR (95% CI)[b]
Smoking status				
Never	24 (13.9)	6 (7.5)	1.00	1.00
Former	100 (57.8)	37 (46.3)	1.66 (0.70–3.93)	1.36 (0.56–3.29)
Current	49 (28.3)	37 (46.3)	2.65 (1.12–6.31)	1.91 (0.79–4.62)
Treatment regimen				
Placebo	88 (50.9)	46 (57.5)	1.00	1.00
13 cRA	85 (49.1)	34 (42.5)	0.83 (0.53–1.29)	0.82 (0.51–1.33)
IGF-I (ng/ml)				
$<$ 104.25	98 (57.7)	25 (31.6)	1.00	1.00
$\geq$ 104.25	72 (42.3)	54 (68.4)	2.66 (1.65–4.28)	2.78 (1.62–4.77)
IGFBP-3 (ng/ml)				
$\geq$ 2276, $<$ 4140	91 (53.2)	32 (40.5)	1.00	1.00
$<$ 2276	74 (43.3)	32 (40.5)	1.07 (0.65–1.75)	1.65 (0.96–2.84)
$\geq$ 4140	6 (3.5)	15 (19.0)	2.78 (1.50–5.15)	2.17 (1.14–4.13)

[a] SPT, second primary tumor; HR, hazard ratio; CI, confidence interval; 13 cRA, 13-cis-retinoic acid; IGF-I, insulin-like growth factor-I; IGFBP-3, IGF binding protein 3.

[b] Cox proportional hazard model was built to assess the prognostic effects of IGF-I and IGFBP-3 on the development of SPT. The model simultaneously includes IGF-I, IGFBP-3, smoking status at registration, and treatment regimen.

risk of SPTs compared with never smokers with a hazard ratio of 1.91 (95% CI, 0.79–4.62). Similar results were found for smoking-related SPTs (Table 5).

DISCUSSION

The purpose of this study was to investigate the relationship between prediagnostic serum levels of IGF-I and IGFBP-3, and subsequent risk for SPT. We found that higher levels of IGF-I and lower and higher levels of IGFBP-3 were indeed associated with significantly increased risk of SPT development.

Animal experiments indicate that overexpression of IGF-I increases the likelihood of tumor development (38). Subsequent prospective and retrospective epidemiologic studies have confirmed this finding demonstrating that elevated serum IGF-I levels are associated with increased risk of various epithelial cancers including prostate, breast, colorectal, and lung cancers (23–34). Recently in a breast cancer clinical trial, a decline in IGF-I serum levels was found to be associated with a lower occurrence of second primary cancers in premenopausal women (39).

The role of IGF-I in the development of cancer is biologically plausible. IGFs enhance proliferation, resistance of damaged cells to apoptosis, and clonal outgrowth of genetically damaged populations leading to tumorigenesis. They increase DNA synthesis and stimulate the expression of cyclin D1, which accelerates the progression of the cell cycle from G_1 to S phase (40). They also have the ability to inhibit apoptosis by modulating the expression of Bcl and Bax, thereby increasing the amount of Bcl/Bax heterodimers, which are required for the initiation of apoptosis (41). Limited evidence suggests that the effects of IGF-I might also be related to *p53* mutations. These mutations are quite common in HNSCCs (42), with reported incidences varying from 43 to 96%. Certain types of *p53* mutations will lead to p53 overexpression, which in turn

Table 5 Cox proportional hazards model of time to development of smoking-related SPTs[a,b]

	No SPT (n = 173)	SPT (n = 58)	Univariate HR (95% CI)	Multivariate HR (95% CI)[c]
Smoking status				
Never	24 (13.9)	4 (6.9)	1.00	1.00
Former	100 (57.8)	26 (44.8)	1.77 (0.62–5.07)	1.54 (0.52–4.51)
Current	49 (28.3)	28 (48.3)	3.12 (1.09–8.92)	2.30 (0.79–6.72)
Treatment regimen				
Placebo	88 (50.9)	35 (60.3)	1.00	1.00
13 cRA	85 (49.1)	23 (39.7)	0.72 (0.43–1.23)	0.81 (0.46–1.43)
IGF-I (ng/ml)				
$<$ 104.25	98 (57.7)	19 (32.8)	1.00	1.00
$\geq$ 104.25	72 (42.3)	39 (67.2)	2.77 (1.60–4.81)	2.50 (1.32–4.72)
IGFBP-3 (ng/ml)				
$\geq$ 2276, $<$ 4140	91 (53.2)	25 (43.1)	1.00	1.00
$<$ 2276	74 (43.3)	20 (34.5)	0.87 (0.48–1.57)	1.32 (0.69–2.54)
$\geq$ 4140	6 (3.5)	13 (22.4)	3.27 (1.67–6.42)	2.57 (1.26–5.23)

[a] SPT, second primary tumor; HR, hazard ratio; CI, confidence interval; 13 cRA, 13-cis-retinoic acid; IGF-I, insulin-like growth factor-I; IGFBP-3, IGF binding protein 3.

[b] Smoking-related SPTs include larynx, oral cavity, pharynx, lung, and bladder tumors.

[c] Cox proportional hazard model was built to assess the prognostic effects of IGF-I and IGFBP-3 on the development of smoking-related SPT. The model simultaneously includes IGF-I, IGFBP-3, smoking status at registration and treatment regimen.

will up-regulate IGF-IR expression (43). The increased expression of IGF-IR will allow for more IGF-I/IGF-IR binding, thereby modulating cell proliferation. It is believed that IGF-I bioactivity in the tissue may determine the turnover rate of epithelial cell populations (44). Therefore, cells with high levels of IGF-I may carry a slightly higher probability of survival than those exposed to normal IGF-I levels (45–46).

Spitz *et al.* (47) reported that *in vitro* sensitivity to bleomycin could be a predictor of tumor recurrence in HNSCC patients. We also demonstrated that there was a joint effect between IGF-I and bleomycin-induced mutagen sensitivity in lung cancer risk (48). Our results suggest that the accumulation of genetic damage may be dependent on an individual's intrinsic sensitivity to carcinogens as well as on humoral factors.

The association between IGFBP-3 and cancer risk is controversial. Several epidemiological studies noted increased risk for colorectal cancer (26, 29–30), breast cancer (23, 25, 49), and prostrate cancer (50) for patients with the highest levels of IGFBP-3. However, in only two of these studies were the risks statistically significant (25, 49). In a case–control study nested in the β-Carotene and retinol efficacy trial cohort, Spitz *et al.* (51) found elevated risks for lung cancer in the highest quartile of IGFBP-3 level with an OR of 2.35 (95% CI, 1.13–4.92). A meta-analysis of prostate cancer and IGF-I levels reported that elevated IGFBP-3 was a significant risk factor for prostate cancer, with an OR of 1.26 (95% CI, 1.03–1.51; Ref. 52). Conversely, several other epidemiological studies have suggested that higher levels of serum IGFBP-3 are significantly associated with a reduced risk for various cancers (27–28, 31–32, 41, 43). In our previous retrospective case control study of IGF-I and IGFBP-3 in lung cancer, we found that higher levels of serum IGFBP-3 were associated with a reduced risk of the disease after adjustment for IGF-I level (31). However, in most of these studies, the associations were weak and not statistically significant. The dual function of IGFBP-3 may explain these contradictory results. Circulating IGFBP-3 modulates the amount of bioavailable free IGF and inhibits its transfer from the circulation to tissue sites of action by competitively binding to IGFs, thereby preventing their binding to IGF-IR and suppressing cell proliferation (53–54). IGFBP-3, in this respect, plays a protective role. IGFBP-3 can also enhance IGF activity by presenting and slowly releasing IGF-I for receptor interactions while protecting the receptor from down-regulation by high IGF-I exposure (54). In this case, IGFBP-3 serves as a risk factor. Interestingly, we found that both the lower and higher levels of IGFBP-3 were associated with an increased risk of SPT. Thus, our results support a dual role function of IGFBP-3. At lower levels of IGFBP-3, most of IGFs are free and can promote cell proliferation through binding to IGF-IR; therefore, lower levels of IGFBP-3 might be a risk factor. At higher levels, IGFBP-3 can bind to IGF-I and enhance its proliferative effect through protecting the receptor from down-regulation by high IGF-I levels. In this case, a higher level of IGFBP-3 also exerts a potential adverse effect. This nonlinear association was uncovered by a computer-intensive preliminary exploratory analysis using recursive partitioning. The actual change points in the pattern of IGFBP-3 underwent a thorough sensitivity analysis based on 500 bootstrap simulations, from which the chosen cutpoints corresponded to those with the highest frequency in the empirical bootstrap distribution.

There are several strengths to this nested case–control study. The first is that the cases and controls were chosen from the same well-defined population of early-stage, upper-aerodigestive tract cancer patients, avoiding selection bias. Secondly, blood samples were obtained before a second tumor developed. The median duration between blood drawn and SPT development was ~32 months (range, 6–69 months). Therefore, it could be said that the high levels of IGF-I and IGFBP-3 could contribute to, rather than be the effect of, the cancer. Possible limitations to this study include the use of a single measure to categorize participants. However, London *et al.* (32) found that the blood samples drawn 0.75–4.75 years apart were still highly correlated ($r = 0.73$) for both IGF-I and IGFBP-3.

To our knowledge this is the first epidemiological study to investigate the association of prediagnostic serum levels of IGF-I and IGFBP-3 as predictors of the risk of developing SPT. Our results indicate that elevated serum level of IGF-I and extremely low or high levels of IGFBP-3 may be important factors to consider in the etiology of SPT. Nevertheless, additional studies are warranted to assess the utility of measuring serum levels of IGF-I and IGFBP-3 in SPT risk. Our results have clinical and prognostic implications for patient surveillance and early detection.

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