Serum Insulin-like Growth Factors, Insulin-like Growth Factor-binding Protein-3, and Risk of Lung Cancer Death: A Case-control Study Nested in the Japan Collaborative Cohort (JACC) Study

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To elucidate the roles of insulin-like growth factors (IGFs) in the development of lung cancer, we conducted a case-control study nested within the Japan Collaborative Cohort Study. Serum samples were collected at baseline from 39 140 men and women between 1988 and 1990. We measured serum IGF-I, IGF-II, and IGF-binding protein-3 (IGFBP-3) in 194 case subjects who subsequently died from lung cancer during an 8-year follow-up and in 9351 controls. The odds ratios (ORs), adjusted for smoking and other covariates, were smaller with higher levels of IGF-II and IGFBP-3. The ORs across quartiles were 0.41 (95% confidence interval [CI], 0.27–0.63), 0.47 (0.31–0.71), and 0.67 (0.46–0.98) for IGF-II (trend $P=0.018$), and 0.55 (95% CI, 0.37–0.81), 0.54 (0.36–0.82), and 0.67 (0.45–1.01) for IGFBP-3 (trend $P=0.037$). These peptides were not independently related to lung cancer risk when mutually adjusted. The risk was increased in the highest vs. the lowest quartile of IGF-I only after controlling for IGFBP-3 (OR, 1.74; 95% CI, 1.08–2.81). Limiting subjects to those followed for ≥3 years strengthened the negative associations of IGF-II and IGFBP-3, whereas the ORs for IGF-I generally decreased. A higher level of circulating IGFBP-3 and/or IGF-II may decrease lung cancer risk. Elevated serum IGF-I may increase the risk, but this could partly be attributable to latent tumors.

Key words: Lung cancer — Insulin-like growth factor-I — Insulin-like growth factor-II — Insulin-like growth factor-binding protein-3 — Nested case-control studies

Insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs) have been investigated in relation to the risk of various cancers.[1] IGF-I and IGF-II may promote the development of lung cancer by stimulating cell proliferation. *In vitro* studies have shown that IGF-I[2, 3] and IGF-II[4] are potent mitogens for lung cancer cells. IGFBP-3, the principal blood IGFBP, binds more than 90% of the IGFs in circulation, thereby restricting the biological activity of IGFs.[5] IGFBP-3 also inhibits cellular proliferation and induces apoptosis through binding to IGFBP-3 receptors.[5, 6]

Increased IGFs and decreased IGFBP-3, therefore, may play a role in the development of lung cancer. There is considerable between-person variability in blood levels of IGF-I, IGF-II, and IGFBP-3,[1, 7, 8] and their levels could possibly predict the risk of lung cancer.

Nevertheless, epidemiological evidence for lung cancer remains insufficient and inconsistent compared with that on cancers of the breast, colon and rectum, and prostate.[1] One case-control study has reported that higher plasma IGF-I and lower plasma IGFBP-3 levels were associated with lung cancer risk.[9] A prospective study[10] failed to confirm these findings, but higher serum levels of IGFBP-3 were inversely related to the risk in a recent cohort study.[11]

We therefore sought to determine, in a prospective study, the association of blood levels of IGF-I, IGF-II, and IGFBP-3 with the risk of death from lung cancer.

MATERIALS AND METHODS

Study population and serum samples We carried out a nested case-control study as a part of the Japan Collaborative Cohort (JACC) Study, sponsored by Monbukagakusho (the Ministry of Education, Culture, Sports, Science and Technology of Japan).[12, 13] This study involves 110 792 residents who were 40–79 years of age at baseline from 45 areas all over Japan. An epidemiological survey on life-style factors was conducted using a self-administered questionnaire, including smoking habits from 1988 to 1990.

Those survey participants who underwent health-screening checks sponsored by municipalities were asked to donate blood samples during the same period as the questionnaire survey. Eventually, 39 140 subjects (35.3% of the respondents to the questionnaire survey) provided blood samples. Sera were separated from the samples at laboratories in or near the municipalities as soon as possible after the blood was drawn. The serum derived from each participant's sample was divided into three to five tubes (100–500 μl per tube), and the tubes were stored in deep freezers at −80°C until analyzed.

Informed consent was obtained from all participants. The Ethical Board of the Nagoya University School of Medicine approved this study.

Case ascertainment and control selection Vital status of the subjects was determined using resident registration records in the relevant municipalities, and causes of death were confirmed from death certificates. We estimated the follow-up rate to be 97% during the study period through the end of 1997 (mean follow-up period±SD=8.1±1.8 years).[12] Incident cases of cancer could be identified by linkage with cancer registries in 24 study areas out of 45.

In the JACC Study, we measured serum levels of IGFs and IGFBP-3 in all cases of deaths and known incident cancers and in their controls to elucidate the association of IGF-related factors with various sites of cancer and other diseases. By the end of 1997, 2134 deaths from all causes in all areas and 733 incident cases of cancer (excluding deaths) in the selected areas were identified among the subjects with baseline serum samples. Three or four controls were randomly selected from all non-cases by matching for study area, gender, and age (as near as possible) for each case. The control group was comprised of 10 351 men and women.

Of the 2867 cases mentioned above, the present analysis was restricted to 196 subjects who died from lung cancer (International Statistical Classification of Diseases and Related Health Problems, 10th Revision: C34)[14] because incident cases of the malignancy were identified only in the selected study areas. We excluded two subjects with a previous history of lung cancer, resulting in 194 cases in

the present study. Since cell types of lung cancer were seldom noted on death certificates, we did not collect the histological information on the certificates and analyzed all cases of lung cancer death as a whole.

In this analysis, we did not limit the controls to those originally matched to the cases of lung cancer death, but attempted to include all the available 10 351 controls to obtain steadier estimates of odds ratios (ORs). We dropped four controls because of inadequate sera. A further 996 controls were excluded because no cases of lung cancer death were found in their area and gender strata. For example, if no women died from lung cancer in one study area, all female controls in that area were eliminated. This left 9351 controls eligible for the present analysis.

Biochemical assays of sera The trained staff, blinded to case-control status, assayed all samples at a single laboratory (SRL, Hachioji) in 1999 and 2000. Serum levels of IGF-I, IGF-II, and IGFBP-3 were measured by immunoradiometric assay, using commercially available kits (Daiichi Radioisotope Lab., Tokyo). Total IGF-I and IGF-II can be measured with the kits by separating IGFs from their binding proteins. The manufacturer has validated the IGF assays against the methods using acid-ethanol extraction and/or acid-column chromatography.

In a pilot study prior to the measurement of cohort samples, the stability of standard curves was examined, as were the sensitivity and reproducibility of the assays. The range for reliable measurement was 4.0–2000 ng/ml for IGF-I, 10–1640 ng/ml for IGF-II, and 0.07–10.10 μg/ml for IGFBP-3; the intra- and inter-assay coefficients of variation were 2.15–3.53% and 1.21–4.11%, respectively, for IGF-I; 2.74–4.45% and 4.23–5.53% for IGF-II; and 3.16–4.19% and 5.28–8.89% for IGFBP-3.

Statistical analysis Body mass index (BMI) at baseline was calculated based on the height and weight reported in the questionnaire survey (BMI=weight in kilograms/[height in meters]^2). We compared baseline characteristics between cases and controls by the χ^2 test or the Mantel test.[15] The cross-sectional relationships among age, BMI, and serum IGF-I, IGF-II and IGFBP-3 were examined using the Spearman correlation coefficient.

ORs were used to relate the risk of lung cancer death to the IGF variables. Since we included not only the controls matched to cases of lung cancer death but also those matched to other cases, the original individual matching (1:3 or 1:4) was not retained. However, we still had *m:n* matching for study area and gender. This refers to the situation where there were a varying number of cases and controls in area- and gender-matched sets. Conditional logistic regression models[16] with area and gender strata, therefore, were applied to calculate ORs for lung cancer death. Age was originally matched as closely as possible between each case and its corresponding controls. Thus, we had no age strata once the individual matching was

destroyed. Accordingly, we included age as a continuous independent variable in the logistic models. We also estimated the ORs using only the 772 controls individually matched to the cases of lung cancer by conditional logistic regression[16] and compared the findings with those from the above-mentioned approach.

The ORs were computed according to quartile levels of IGF-I, IGF-II, and IGFBP-3. To consider variations between study areas, cut-off points for quartiles were determined according to the distribution of controls in each area. The control subjects, however, were not precisely divided into four even groups due to identical measurement values of IGFs and IGFBP-3. To test for linear trends in ORs over quartiles, we assigned the median value for each category, and then incorporated the score into the logistic models as a single variable.[17]

We also considered potential confounding by smoking habits (never, former, and current smokers, and unknown) and BMI (<20.0, $20.0–24.9$, ≥25.0 [kg/m^2], and unknown). To account for possible confounding by current or past smoking in more detail, smoking was also adjusted using finer strata. The strata used in the logistic models were as follows: non-smokers, ex-smokers who had quit 0–4, 5–9, 10–14, 15–19, and ≥20 years ago, current smokers with 0–19, 20–39, 40–59, 60–79, 80–99, and ≥100 pack-years, and unknown. All P values were two-sided and all statistical analyses were performed using the Statistical Analysis System.[18]

RESULTS

Table I shows baseline characteristics of cases of lung cancer death and controls. The mean time$\pm$SD between blood collection and death from lung cancer in cases was 5.2 ± 2.2 years. Cases were older and included a higher proportion of men than controls. This is because the controls who were initially matched to cases other than lung cancer death but were included in this analysis were younger and more likely to be women than those who were originally matched to cases of lung cancer death. As expected, the proportion of ex-smokers or current smokers was higher in cases of lung cancer death than in controls. Controls tended to have a higher BMI than cases.

The mean$\pm$SD (median) values of serum IGF-I, IGF-II, and IGFBP-3 were 124 ± 54 ng/ml (120 ng/ml), 548 ± 137 ng/ml (530 ng/ml), and 2.78 ± 0.86 μg/ml (2.68 μg/ml) in cases of lung cancer death, respectively. The corresponding figures were 126 ± 57 ng/ml (120 ng/ml), 586 ± 126 ng/ml (580 ng/ml), and 2.99 ± 0.81 μg/ml (2.92 μg/ml) in controls.

Table II shows the interrelationships among age, BMI, IGF-I, IGF-II, and IGFBP-3 in the controls. Serum IGF-I and IGF-II levels were positively correlated (Spearman's $r=0.43$), and both IGFs showed even closer correlations

with IGFBP-3 ($r=0.56$ for IGF-I and 0.77 for IGF-II). IGF-I, IGF-II, and IGFBP-3 were somewhat positively associated with BMI, but inversely correlated with age.

Table III summarizes the ORs for lung cancer death by serum levels of IGF-I, IGF-II, and IGFBP-3. For all the subjects (the left half of Table III), the ORs adjusted for study area, gender, age, smoking habits, and BMI (OR1) were smaller with higher levels of IGF-II or IGFBP-3. The ORs across quartiles were 0.41, 0.47, and 0.67 for IGF-II (P for trend=0.018) and 0.55, 0.54, and 0.67 for IGFBP-3 (P for trend=0.037).

Because IGFBP-3 regulates the action of IGFs,[1, 5] we evaluated the association of IGF-I and IGF-II with the risk

Table I. Distribution of Cases of Lung Cancer Death and Controls by Baseline Characteristics

Characteristic	Cases		Controls		P for difference
	n	%	n	%	
Gender					
Men	148	76.3	5187	55.5	
Women	46	23.7	4164	44.5	<0.001
Age (years)					
40–49	7	3.6	657	7.0	
50–59	29	14.9	1936	20.7	
60–69	92	47.4	4589	49.1	
70–79	66	34.0	2169	23.2	<0.001
Smoking habits					
Never smokers	38	19.6	4761	50.9	
Ex-smokers	39	20.1	1552	16.6	
Current smokers	110	56.7	2645	28.3	
Unknown	7	3.6	393	4.2	<0.001
Body mass index (kg/m^2)					
<20.0	37	19.1	1486	15.9	
20.0–24.9	123	63.4	5519	59.0	
≥25.0	22	11.3	1777	19.0	
Unknown	12	6.2	569	6.1	0.011

Table II. Spearman Correlation Coefficients between Age, BMI, and Serum IGF-I, IGF-II and IGFBP-3 among Controls ($n=9351$)

	Age	BMI	IGF-I	IGF-II
BMI	−0.11			
IGF-I	−0.26	0.08		
IGF-II	−0.18	0.17	0.43	
IGFBP-3	−0.22	0.16	0.56	0.77

BMI, body mass index; IGF-I, insulin-like growth factor-I; IGF-II, insulin-like growth factor-II; IGFBP-3, insulin-like growth factor-binding protein-3. All the correlation coefficients were statistically significant ($P<0.001$).

Table III. Odds Ratios for Death from Lung Cancer by Serum Levels of IGF-I, IGF-II, and IGFBP-3

Quartile	All subjects								Subjects followed for 3 years or more							
	Cases		Controls[a]		OR1[c]	95% CI	OR2[d]	95% CI	Cases		Controls[b]		OR1[c]	95% CI	OR2[d]	95% CI
	n	%	n	%					n	%	n	%				
IGF-I																
1st quartile	44	22.7	2122	22.7	1.00		1.00		43	27.2	2128	22.9	1.00		1.00	
2nd quartile	49	25.3	2192	23.4	1.04	0.69–1.59	1.28	0.83–1.97	40	25.3	2167	23.3	0.87	0.56–1.36	1.08	0.68–1.70
3rd quartile	38	19.6	2443	26.1	0.73	0.46–1.14	1.01	0.62–1.63	29	18.4	2366	25.4	0.58	0.36–0.94	0.82	0.49–1.37
4th quartile	63	32.5	2594	27.7	1.17	0.78–1.77	1.74	1.08–2.81	46	29.1	2650	28.5	0.84	0.54–1.31	1.32	0.78–2.21
					P for trend=0.61		*P* for trend=0.043						*P* for trend=0.31		*P* for trend=0.41	
IGF-II																
1st quartile	81	41.8	2198	23.5	1.00		1.00		71	44.9	2185	23.5	1.00		1.00	
2nd quartile	32	16.5	2230	23.8	0.41	0.27–0.63	0.48	0.30–0.76	28	17.7	2216	23.8	0.42	0.27–0.65	0.49	0.30–0.81
3rd quartile	34	17.5	2373	25.4	0.47	0.31–0.71	0.56	0.33–0.95	26	16.5	2365	25.4	0.41	0.26–0.66	0.52	0.29–0.94
4th quartile	47	24.2	2550	27.3	0.67	0.46–0.98	0.77	0.43–1.40	33	20.9	2545	27.3	0.54	0.35–0.84	0.71	0.37–1.38
					P for trend=0.018		*P* for trend=0.31						*P* for trend=0.001		*P* for trend=0.19	
IGFBP-3																
1st quartile	77	39.7	2298	24.6	1.00				68	43.0	2297	24.7	1.00			
2nd quartile	40	20.6	2342	25.0	0.55	0.37–0.81			33	20.9	2321	24.9	0.51	0.33–0.78		
3rd quartile	36	18.6	2339	25.0	0.54	0.36–0.82			30	19.0	2329	25.0	0.51	0.33–0.80		
4th quartile	41	21.1	2372	25.4	0.67	0.45–1.01			27	17.1	2364	25.4	0.50	0.31–0.80		
					P for trend=0.037								*P* for trend=0.002			

OR, odds ratio; CI, confidence interval; IGF-I, insulin-like growth factor-I; IGF-II, insulin-like growth factor-II; IGFBP-3, insulin-like growth factor-binding protein-3. Subjects were categorized according to the quartiles of serum IGF-I, IGF-II, and IGFBP-3 among controls in each study area. Controls were not precisely divided into four even groups due to identical measurement values of IGFs and IGFBP-3.
a) Median measurement values across the quartiles were 79, 110, 140, and 180 ng/ml for IGF-I, 440, 540, 610, and 710 ng/ml for IGF-II, and 2.13, 2.68, 3.13, and 3.81 µg/ml for IGFBP-3.
b) Median measurement values across the quartiles were 80, 110, 140, and 180 ng/ml for IGF-I, 440, 540, 610, and 710 ng/ml for IGF-II, and 2.13, 2.68, 3.13, and 3.81 µg/ml for IGFBP-3.
c) Adjusted for area, gender, age, smoking habits, and body mass index.
d) Adjusted for area, gender, age, smoking habits, body mass index, and IGFBP-3.

of lung cancer death by further adjustment for the quartiles of IGFBP-3 (OR2). IGF-II was not related to the risk independently of IGFBP-3. On the contrary, the risk was elevated in the highest quartile compared with the lowest quartile of IGF-I (OR2, 1.74; 95% confidence interval [CI], 1.08–2.81).

At the same time, adjusting for IGF-I levels enhanced the risk reduction by higher IGFBP-3. The ORs, moving from the second to the top quartile of IGFBP-3, were 0.51 (95% CI, 0.34–0.76), 0.47 (0.30–0.73), and 0.53 (0.33–0.84) (*P* for trend=0.007, adjusted for area, gender, age, smoking, BMI, and IGF-I). The decrease in risk with increasing IGFBP-3, however, turned out to be insignificant when adjusted for IGF-II. The ORs across quartiles were 0.75 (95% CI, 0.48–1.17), 0.76 (0.44–1.32), and 0.82 (0.44–1.53) (*P* for trend=0.55, adjusted for area, gender, age, smoking, BMI, and IGF-II).

To exclude possible effects of latent lung cancer on IGF and IGFBP levels, we limited the analysis to those followed for at least 3 years (158 cases and 9311 controls; the right half of Table III). This analysis strengthened the negative associations of IGF-II and IGFBP-3 with the risk of lung cancer death. The OR1 over increasing quartiles were 0.42, 0.41, and 0.54 for IGF-II (*P* for trend=0.001) and 0.51, 0.51, and 0.50 for IGFBP-3 (*P* for trend=0.002). IGF-II (OR2) and IGFBP-3 (data not shown) were not independently associated with the risk when mutually adjusted (*P* for trend=0.19 for IGF-II and 0.24 for IGFBP-3).

The ORs for IGF-I generally decreased in this restricted analysis compared with those in all the subjects. The IGFBP-3-adjusted OR (OR2) for the highest quartile vs. the lowest quartile decreased to 1.32 (95% CI, 0.78–2.21). The negative association between IGFBP-3 and the risk remained essentially unchanged after adjustment for IGF-I. The ORs across quartiles were 0.51 (95% CI, 0.33–0.79), 0.49 (0.30–0.79), and 0.45 (0.26–0.77) (*P* for trend=0.003, adjusted for area, gender, age, smoking, BMI, and IGF-I).

The more detailed adjustment for smoking habits stratifying ex-smokers and current smokers as mentioned above hardly altered the association of IGFs or IGFBP-3 with the risk of lung cancer death. The ORs in Table III changed only by 0.03 at the most (data not shown). The ORs remained almost the same after adjustment for the year of the baseline survey, which implies minimal effects of the sampling date. The ORs changed only by 0.02 or less.

When the ORs were estimated using only the individually matched controls, a decreased risk associated with the higher serum levels of IGF-II and IGFBP-3 remained unchanged. On the other hand, the risk of lung cancer death was not elevated in the highest quartile of serum IGF-I even after controlling for IGFBP-3.

The ORs adjusted for study area, gender, age, smoking habits, and BMI across quartiles were 0.69 (95% CI, 0.43–1.11), 0.66 (0.42–1.06), and 0.80 (0.51–1.26) for IGF-I (*P* for trend=0.52); 0.48 (0.30–0.76), 0.58 (0.37–0.92), and 0.54 (0.34–0.87) for IGF-II (*P* for trend= 0.020); and 0.69 (0.44–1.08), 0.53 (0.33–0.85), and 0.54 (0.33–0.86) for IGFBP-3 (*P* for trend=0.007). IGF-I and IGF-II were not related to the risk independently of IGFBP-3. The ORs adjusted for area, gender, age, smoking, BMI, and IGFBP-3, moving from the second to the top quartile, were 0.81 (95% CI, 0.49–1.34), 0.84 (0.51–1.38), and 1.16 (0.69–1.97) for IGF-I (*P* for trend= 0.40) and 0.55 (0.33–0.91), 0.81 (0.45–1.44), and 0.84 (0.43–1.63) for IGF-II (*P* for trend=0.66).

The risk reduction by higher IGFBP-3 was independent of IGF-I levels. The IGF-I-adjusted ORs across quartiles were 0.69 (95% CI, 0.43–1.10), 0.52 (0.32–0.87), and 0.49 (0.28–0.85) (*P* for trend=0.009, adjusted for area, gender, age, smoking, BMI, and IGF-I). On the contrary, the decrease in risk with increasing IGFBP-3 was attenuated when adjusted for IGF-II. The corresponding ORs were 0.83 (95% CI, 0.50–1.37), 0.61 (0.33–1.11), and 0.56 (0.28–1.12) (*P* for trend=0.10, adjusted for area, gender, age, smoking, BMI, and IGF-II).

DISCUSSION

In this prospective study, we observed a decrease in the risk of lung cancer death in relation to higher serum concentrations of IGF-II and IGFBP-3. Although the risk unadjusted for IGFBP-3 was not associated with serum IGF-I levels, those with higher IGF-I had an elevated risk after controlling for IGFBP-3.

It may not be a typical approach to include not only the controls initially matched to cases of lung cancer death, but also those matched to other cases. This procedure, however, corresponds to sampling controls according to the distribution of all the cases (i.e., all deaths and known incident cancers) by study area, gender, and age, and whether a non-case subject was selected as a control or not

was solely dependent on his or her area, gender, and age. The OR estimates, therefore, were not biased after the appropriate adjustment for these three variables in the logistic models. Inclusion of all the available controls enabled us to obtain more stable estimates of ORs than using only the controls originally matched to cases of lung cancer death.

Excluding cases other than lung cancer death from the source of controls might have biased the results,[19] but such cases accounted for a small proportion (6.8%) of subjects with baseline serum samples. Furthermore, even when all cases other than lung cancer were included in the analysis *as controls*, our overall conclusions remained virtually unchanged.

The prospective design of our study minimized potential bias due to systematic case-control differences in blood collection. Although we obtained serum samples only at baseline, some studies[20, 21] have reported that blood levels of IGF-I and IGFBP-3 have relatively small within-individual variation over time. Thus, single measures should provide a reasonable indicator of a subject's usual blood levels for these growth factors.

Some methodological issues, however, should be kept in mind when interpreting the findings. First, the end point of this investigation was not the incidence of lung cancer, but the mortality due to lung cancer. The control series might have included some lung cancer survivors, causing an attenuation of the associations of IGF-related factors with the risk of lung cancer. Nevertheless, the proportion of lung cancer survivors in controls should be very small, considering the incidence rate (45.5 to 70.0 in men and 17.3 to 25.9 in women per 100 000 population) together with the high death-to-incidence ratio (0.71 to 0.87) of lung cancer in Japan.[22] We could not validate the diagnosis on death certificates and some misclassification of the disease may have occurred. It is likely that the misclassification is independent of serum IGF or IGFBP-3 levels at baseline. The ORs, therefore, would have been somewhat biased toward the null.[23]

Second, the serum samples had been stored in deep freezers for about 10 years until assayed. Unfortunately, we could not directly examine the change in levels of IGFs and IGFBP-3 in the cohort samples because these components were not measured at the time of blood collection. The mean serum IGF-I, IGF-II, and IGFBP-3 in controls (*n*=9351), however, differed only by 5.3–7.6% from the weighted means in fresh samples derived from a healthy Japanese population (*n*=318; means weighted by the distribution of age and sex in controls).[24] The fresh specimens were assayed with the same kits and procedures as ours. The small difference between the two sets of means, therefore, implies that the serum levels of IGF-related factors remained reasonably stable during long-term storage.

Finally, only one-third (35.3%) of the participants in the questionnaire survey gave blood samples. This might reduce the external validity of our study. Nevertheless, the cases and controls derived from the subjects with sera are still comparable because the two groups underwent the same selection process before providing blood samples.

Higher levels of serum IGF-II and IGFBP-3 were associated with a lower risk of lung cancer in our study, particularly among the subjects followed for 3 years or more. The two molecules, however, were not independently related to the risk when mutually adjusted. As in other studies,[9, 25, 26] IGF-II had a strong positive correlation with IGFBP-3. Therefore, it may be difficult to determine the independent effects of the two peptides even by using multivariate models. The trends in risk associated with serum levels of IGF-II and IGFBP-3 should be studied further because the lowest ORs were often found in the second or third quartiles. The statistical significance of trend tests, in part, might have emerged from the large number of controls.

We speculate, however, that IGFBP-3 would be relevant to the reduced risk of lung cancer because of its anticarcinogenic activities.[5, 6] The effects of two antiproliferative molecules against lung cancer, wild-type p53 protein[27] and retinoic acid,[28, 29] may also be mediated, at least in part, by stimulating the production of IGFBP-3. Yu *et al.*[9] reported a negative association of plasma IGFBP-3 with the risk of lung cancer in a case-control study after adjustment for IGF-I. Although their observations were not supported by a prospective study by Lukanova and co-workers,[10] London *et al.* found a reduced risk in subjects with higher serum levels of IGFBP-3 in a male cohort. It is interesting that decreases in the risk of prostate,[20] colorectal,[30] and breast[31] cancers have been related to elevated circulating levels of IGFBP-3.

The risk reduction by IGF-II in our study seems strange because IGF-II acts as a mitogen through the IGF-I receptor.[1, 32] The study by Yu *et al.* did not show an association of IGF-II with a decreased risk of lung cancer.[9] Further investigations, however, are warranted since some studies have found a reduction in the risk of prostate[33] and breast[26] cancers associated with higher levels of IGF-II.

The case-control study by Yu and co-workers[9] reported a positive association between IGF-I and lung cancer risk. The top vs. bottom quartile OR adjusted for IGFBP-3 was as high as 2.75. Furthermore, many epidemiological investigations have provided reasonably consistent support for an increased risk of solid tumors other than lung cancer in relation to higher levels of IGF-I.[1] However, two prospective studies of lung cancer[10, 11] failed to replicate the findings by Yu *et al.*[9]

We found an enhanced risk of lung cancer in the highest IGF-I level when controlling for IGFBP-3. Adjustment for IGFBP-3 levels strengthened the association of IGF-I also

in the preceding study.[9] The greater OR, however, somewhat decreased when we limited the present analysis to subjects with at least a 3-year follow-up. This reduction in risk may partly be explained by latent lung cancer at baseline since the tumor itself produces IGF-I and may elevate blood levels.[2, 3] Another explanation may be that the intra-individual variation of circulating IGF-I, though relatively small,[20, 21] attenuated the association during the long follow-up. Possible residual confounding should also be considered because the analysis involving only the individually matched controls did not detect the increased risk.

In our study, histological information was available for only a quarter of cases ($n=48$) from cancer registries, which precluded us from estimating ORs by histological type. The distributions of serum IGF-I, IGF-II, and IGFBP-3 in cases of squamous cell carcinoma ($n=21$) did not significantly differ from those in adenocarcinoma cases ($n=17$) (all $P>0.20$, by Mann-Whitney test), but this may be ascribed to the small sample size. Lukanova *et al.*[10] reported that limiting their analysis to adenocarcinoma did not influence the associations of IGF-I and IGFBP-3 with lung cancer risk. London and co-workers[11] found a reduced risk in subjects with the highest levels of IGFBP-3 irrespective of histological groups. Further investigations on this issue, however, are still needed because of the small number of cases by histological type in these previous studies.

In summary, our results suggest that higher blood IGFBP-3 and/or IGF-II levels may decrease the risk of lung cancer. Those with higher IGF-I had an increased risk when controlling for IGFBP-3, but this could partly be due to latent lung tumors. If our findings are confirmed by further studies, work on the determinants of IGF-related factors in circulation may provide new clues to the prevention of lung cancer.

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