# Additive Interaction Between the Renin-Angiotensin System and Lipid Metabolism for Cancer in Type 2 Diabetes

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**OBJECTIVE—**Clinical and experimental studies suggest crosstalk between lipid metabolism and the renin-angiotensin system (RAS) in atherogenesis. The aim of this study was to explore interactions between these two systems in mediating cancer risk in type 2 diabetes.

**RESEARCH DESIGN AND METHODS—**A prospective cohort of 4,160 Chinese patients with type 2 diabetes, free of cancer at enrollment, were analyzed using Cox models. Interaction of RAS inhibitors (angiotensin I–converting enzyme inhibitors or angiotensin II receptor blockers) and statins was estimated using relative excess risk due to interaction (RERI), attributable proportion due to interaction (AP), and synergy index (S). RERI $> 0$, AP $> 0$, or S $> 1$ indicates additive interaction between the two classes of drugs. Molecular mechanisms underlying these interactions were explored using a uninephrectomy (UNX) rat model with renal carcinogenesis.

**RESULTS—**During 21,992 person-years of follow-up, 190 patients developed cancer. Use of RAS inhibitors and statins in isolation or combination during follow-up was associated with reduced risk of cancer after adjustment for covariates. The multivariable RERI and AP for the additive interaction between these drug classes for cancer were significant (0.53 [95% CI 0.20–0.87] and 2.65 [0.38–4.91], respectively). In the UNX rat model, inhibition of the RAS prevented renal cell carcinoma by normalizing hydroxymethylglutaryl-CoA reductase (HMGCR) expression and the insulin-like growth factor-1 (IGF-1) signaling pathway.

**CONCLUSIONS—**Combined use of RAS inhibitors and statins may act synergistically to reduce cancer risk, possibly via HMGCR and IGF-1 signaling pathways in high-risk conditions such as type 2 diabetes. ***Diabetes*** **58:1518–1525, 2009**

Type 2 diabetes is associated with increased risk of a variety of cancers (1) such as colorectal (2), pancreatic (3), and liver cancers (4), as well as breast (5) and endometrial cancers (6) in women and prostate cancers in men (7). In Hong Kong, type 2 diabetic patients have a 30% increased risk of cancer compared with that of the general population (8). We have previously reported nonlinear relationships between lipids and cancer risks in type 2 diabetes (9). The risk association of cancer with LDL cholesterol was V-shaped, with both LDL cholesterol levels of $<2.80$ mmol/l and $\geq 3.80$ mmol/l being associated with elevated risks of cancer (8).

Large-scale epidemiological studies have suggested that the use of renin-angiotensin system (RAS) inhibitors is associated with a reduced risk of new onset of cancer (10–12), but whether statin use alters cancer risks remains controversial (13,14). Based on our previous findings on the nonlinear relationships between lipids and cancer risk in type 2 diabetes (8,9), we hypothesized that the mevalonate pathway, which leads to cholesterol synthesis, can produce other molecules such as the isoprenoids farnesol and geranylgeraniol and that these small proteins are involved in cell proliferation, differentiation, apoptosis, and thus cancer (8). There is now consistent data from experimental, animal, and human studies suggesting activation of the local/systemic RAS in type 2 diabetes (15). In a retrospective survey, type 2 diabetic patients treated with ACE inhibitors were found to have a lower risk of cancer than those not receiving this drug (16). In support of interactions between dyslipidemia and RAS activation in atherogenesis (17), the combined use of rosuvastatin, a hydroxymethylglutaryl-CoA reductase (HMGCR) inhibitor, and candesartan, an angiotensin receptor blocker, has been shown to have synergistic effects in reducing atherosclerosis in animal studies (18).

Among different growth-promoting pathways, there is emerging evidence suggesting that components of the insulin-like growth factor-1 (IGF-1) system may be implicated in atherogenesis, type 2 diabetes, and cancers. Binding of IGF-1 and insulin to their receptors results in activation of the phosphatidylinositol 3-kinase/Akt signaling pathway and protein kinase (PK) C ζ. IGF-1 and insulin signaling systems have important roles in energy metabolism and cell growth associated with diabetes risk and cancer (19). Interestingly, inhibition of HMGCR activity by statins caused growth arrest via depressing the expression of the functional IGF-1 receptor in multiple cancer cells (20), and thus statins could have therapeutic significance in IGF-1–dependent neoplasms (21,22). In addition, RAS inhibition attenuates IGF-1–induced cardiac fibroblast pro-

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Received 23 January 2009 and accepted 15 April 2009.

Published ahead of print at http://diabetes.diabetesjournals.org on 28 April 2009. DOI: 10.2337/dc09-0105.

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liferation (23) and elevates insulin growth factor–binding protein 3 (IGFBP3) levels among hypertensive older adults (24).

It has long been recognized that nephrectomy in rats (25) and humans (26) leads to compensated remnant kidney growth, proteinuria, and hypertension and is associated with local RAS activation. Recently, we reported the presence of elevated blood glucose and blood lipids, associated with chronic renal impairment and insulin resistance in uninephrectomized rats followed up for 10 months after the operation (27,28). These findings suggest that the uninephrectomy (UNX) rat model may serve as a useful model for the study of metabolic disorders and complications related to type 2 diabetes; including the possible interaction between RAS activation and lipid metabolism for cancer in type 2 diabetes.

Against this background, we hypothesized that combined use of RAS inhibitors and statins is associated with reduced cancer risk in type 2 diabetes and that these clinical benefits may be mediated via modulation of the HMGCR and IGF-1 pathways.

## RESEARCH DESIGN AND METHODS

**Epidemiological analysis.** Details on the methodology of the cohort study have been described previously (8). The Hong Kong Diabetes Registry was established in 1995 and enrolls 30–50 ambulatory diabetic patients per week. Patients were referred by general practitioners and internists from community- and hospital-based clinics or were discharged from the Prince of Wales Hospital or other regional hospitals. Less than 10% of these enrolled patients have had hospital admissions within 6–8 weeks before assessment.

The 4-h assessment of complications and risk factors, modified from the European DiabCare protocol (29), was performed on an outpatient basis. Once a diabetic subject had undergone this comprehensive assessment, he or she was considered to have entered this study cohort and would be observed until death. Ethical approval was obtained from the Clinical Research Ethics Committee of the Chinese University of Hong Kong.

Hospital services in Hong Kong were subsidized to a large extent by the government through the Hospital Authority, the governing body for all publicly funded hospitals and outpatient clinics (30). All patients attending Hospital Authority hospital clinics either as outpatients or inpatients are dispensed medications on site. Clinical end points, including discharge diagnoses of hospital admissions and mortality from 1 January 1995 until 30 July 2005 were used for defining the end points. The Hospital Authority Central Computer System was used to retrieve all hospital admissions and drug-dispensing data. These databases were successfully matched by a unique identification number, the Hong Kong Identity Card number, which is compulsory for all residents in Hong Kong and is used by all government departments and major organizations.

From 1995 to 2005, 7,920 diabetic patients were enrolled in this registry. We limited the analysis to 7,387 patients who were enrolled after 1 December 1996 when dispensing data were computerized. The following exclusion criteria were applied before analysis: diagnosis of type 1 diabetes ($n = 323$) (31), missing data on types of diabetes ($n = 5$), non-Chinese or unknown nationality ($n = 45$), cancer or receiving treatment for cancer at enrollment ($n = 175$), and missing values for variables used in the analysis ($n = 736$) (see footnotes to Table 2 for the variable list). LDL cholesterol is a major confounding factor for cancer in type 2 diabetes (8). Because the pretreatment LDL cholesterol levels were not documented in the registry and use of statins and RAS inhibitors may modify risk associations between LDL cholesterol and cancer, we excluded 827 patients who were using statins and 1,116 patients who were using RAS inhibitors at enrollment to reduce confounding effects due to treatment at baseline. A total of 4,160 patients were entered into the present analysis.

**Definition of end points.** All hospital discharge principal diagnoses including cancer and non–cancer-related hospital admissions were regularly coded by a team of trained personnel under the Hospital Authority, according to ICD-9. Mortality data from the Hong Kong Death Registry were retrieved, and the causes of death were verified against hospital admission records in the Hong Kong Hospital Authority computer system. ICD-9 codes were used to identify first admissions relating to a diagnosis of cancer. The end point of this study was defined as having a first cancer event during the follow-up period (code 140–208), including fatal and nonfatal cancer.

**Clinical and laboratory measurements.** On the day of enrollment, all patients attended the Diabetes and Endocrine Centre of the Prince of Wales Hospital after at least 8 h of fasting and without taking any medication. A sterile, random spot urine sample was used to measure the albumin-to-creatinine ratio (ACR). Albuminuria was defined as ACR ≥2.5 mg/mmol in men and ≥3.5 mg/mmol in women. Total cholesterol, triglycerides, and HDL cholesterol were measured by enzymatic methods on a Hitachi 911 automated analyzer (Boehringer Mannheim, Mannheim, Germany) using reagent kits supplied by the manufacturer of the analyzer. LDL cholesterol was calculated by the Friedewald equation (32). The precision performance of these assays was within the manufacturer's specifications.

**Statistical analyses.** SAS (release 9.10; SAS Institute, Cary, NC) was used to perform all statistical analysis. Cox proportional hazards regression was used to obtain hazard ratios (HRs) with 95% CI. Follow-up time was calculated as the period from enrollment to the date of first admission for cancer, death, or 30 July 2005, whichever came first.

We tested multiplicative and additive interactions between use of RAS inhibitors and statins for cancer. Multiplicative interaction was tested using a term of the product of two variables in Cox models. There are three measures to test additive interaction (33,34): *1*) relative excess risk due to interaction (RERI), *2*) attributable proportion due to interaction (AP), and *3*) synergy index (S). RERI is the excess risk due to interaction relative to the risk without exposure. AP refers to the attributable proportion of disease that is due to interaction among individuals with both exposures. S is the excess risk from both exposures when there is an additive interaction, relative to the risk from both exposures without interaction. RERI $> 0$, AP $> 0$, or S $> 1$ indicates additive interaction. In Cox models, the RERI is the best choice among the three measures (35). A detailed calculation method of additive interaction including definition of three indicator variables, an SAS program, and a calculator in Excel (available at http://www.epinet.se) was described by Andersson et al. (34). Briefly, the three indicator variables were generated for different combinations of exposure to use of statins and use of RAS inhibitors (1 = Yes/0 = No) (see additive interaction models of Table 2 for details). The SAS program delivered estimates of the required parameters together with the covariance matrix, which are used in calculation of the interaction measures in the Excel calculator.

To control for the confounding effects of drug use, we used Yes/No coding, which was a more robust measure than the duration of drug use with or without adjustment for the period of discontinuation (8). A structured adjustment scheme was used to evaluate the additive interaction of the use of statins and that of RAS inhibitors. First, we adjusted for LDL cholesterol–related risk factors, i.e., LDL cholesterol ≥3.80 mmol/l and LDL cholesterol <2.80 mmol/l plus albuminuria (X.Y., W.Y.S., R.C.W.M., et al., unpublished data), age, sex, BMI, and the use of tobacco and alcohol. Second, we further adjusted for metabolic variables (see footnotes to Table 2 for details) and drug use from enrollment to cancer, death, or censoring dates. To avoid overfitting, a propensity score was used to adjust for the covariates, in which a restricted cubic spline with four knots at the 5th, 35th, 65th, and 95th percentiles was used to adjust for the confounding effects of nonlinear associations of lipids and other continuous covariates as before (8). Stratified Cox models on deciles of the propensity score were used in all of the Cox models to adjust for likelihoods of drug use during follow-up or cancer where appropriate (36) (see Table 2 for details). Proportional hazard assumptions of baseline variables and correlations between pairs of baseline variables were also checked as described previously (8). Two-sided $P < 0.05$ was considered to be significant.

**Animal experiments.** We developed a UNX rat model characterized by renal carcinogenesis, RAS activation, and dysmetabolism of glucose and lipids to examine disease mechanisms and drug effects. Details of the experimental protocol and phenotypes were described previously (27,28). Male Sprague-Dawley rats (300–350 g) were obtained from the Laboratory Animal Services Centre at the Chinese University of Hong Kong and maintained at our Research Unit at the Prince of Wales Hospital. The animals were caged in pairs, housed at 22–24°C with a 12-h dark/light cycle and free access to water, and fed a standard laboratory rat diet (5001 Rodent Diet; LabDiet, St. Louis, MO). The total duration of the studies was 10 months.

The animals were randomized into three groups: sham operation ($n = 8$), left UNX ($n = 8$), and UNX rats treated with the ACE inhibitor (ACEI) lisinopril ($n = 8$). Lisinopril were dissolved in 3 ml sterile distilled water, with a once-daily dose of 4 mg/kg body weight. All of the sham and UNX rats were also gavaged with distilled water (3 ml) as a placebo control. Ethical approval for the animal study was obtained from the Animal Experimentation Ethics Committee of The Chinese University of Hong Kong and in accordance to the Animals (Control of Experiments) Ordinance of the Department of Health of the Hong Kong SAR Government.

**Biochemical studies.** At 3, 6, and 8 months after the operation, 24-h urine samples were collected using metabolic cages (Huang Qiao Yin Xing Animal Cage & Equipments Factory, Suzhou, China). When rats were killed 10 months

after the operation, fasting blood samples were taken for the measurement of renal function and lipids including total cholesterol, triglyceride, LDL cholesterol, and HDL cholesterol.

**Histological studies of kidneys.** Histopathological criteria for diagnosis of renal cell carcinoma in the UNX rats included cytological atypia, bizarre nuclei, frequent mitotic figures, and invasive growth. Absence of these morphological characteristics in at least three tissue sections indicated absence of renal cancer. Rats were killed at 10 months after the operation. Kidneys from all of the rats were removed, weighed, and processed for light microscopy. Tissue samples were fixed in 10% neutral formaldehyde and embedded in paraffin. Serial longitudinal sections (4 μm) were spliced parallel to the longest axis of the kidney and stained with periodic acid Schiff. Stained slides were examined with a Zeiss Axioplan 2 imaging microscope (Carl Zeiss, Hamburg, Germany), and representative images were captured using a Spot digital camera.

**Western blot assays.** Tissue total proteins from renal cortex were extracted. The resolved proteins were then transferred onto nitrocellulose membranes. The membranes were blocked for 1 h at room temperature with 5% skimmed milk, incubated with primary antibodies against IGFBP3 (dilution 1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA), Akt1/2/3 (dilution 1:1,000; Cell Signaling Technology, Danvers, MA), and PKCζ (dilution 1:1,000; Santa Cruz Biotechnology) in Tris-buffered saline containing 0.05% Tween 20 (TBS-T) with 5% skimmed milk overnight at 4°C. After washing with TBS-T, membranes were incubated with anti-goat, anti-rabbit, or anti-mouse secondary antibody conjugated to horseradish peroxidase (Upstate Biotechnology, Billerica, MA) with dilution of 1:2,000. Proteins were detected by enhanced chemiluminescence (Amersham, Piscataway, NJ) on Hyperfilm. The major protein bands with ~42 kDa for IGFBP3, 60 kDa for Akt1/2/3, and 80 kDa for PKCζ were detected. To ensure equal loading of proteins, membranes were incubated and probed with a rabbit anti–β-actin antibody (Abcam, Cambridge, MA) with a dilution of 1:10,000, which recognizes β-actin at ~43 kDa. Signals were quantitated by densitometry and corrected for the β-actin signal, using a Kodak Digital Image station 440CF and the ID Image Analysis software program. Treatment groups were compared using ANOVA, and $P < 0.05$ was considered statistically significant.

## RESULTS

**Characteristics of the patients.** The median age of the cohort was 55 years (25th–75th percentiles 45–66 years), with a median duration of diabetes of 5 years (1–10 years). During a total of 21,992 person-years of follow-up, 190 patients developed cancer, giving an annual incidence of 8.64 (95% CI 7.42–9.86) per 1,000 person-years. Patients who subsequently developed cancer were older, were more likely to be smokers and alcohol drinkers, and had a longer duration of diabetes and poorer metabolic profile than those without cancer (Table 1). They were also more likely to use antihypertensive drugs other than RAS inhibitors than patients without cancer. Patients who developed cancer were less likely to be treated with statins alone or statins combined with RAS inhibitors during the follow-up period than those who remained free of cancer.

**Additive interaction between statins and RAS inhibitors.** Compared with nonusers of statins and RAS inhibitors, subjects who were exposed to statins and/or RAS inhibitors had a lower risk of cancer after adjustment for drug use indications and demographic and lifestyle covariates. The additive interaction between statins and RAS inhibitors on cancer risk was significant as indicated by the RERI (0.39 [95% CI 0.09–0.69]) and AP values (1.57 [0.21–2.94]). The statistical significances of use of statins only, use of RAS inhibitors only, combined use of statins and RAS inhibitors, and additive interaction measures (RERI and AP) persisted after adjustment for all of the above factors, metabolic covariates, and use of other drugs at enrollment and during follow-up using a propensity score as well as taking nonlinear associations into account (Tables 2 and 3). Combined use of statins and RAS inhibitors was consistently associated with lower risks of cancer even after removal of 682 patients followed for <2.5 years (HR 0.35 [95% CI 0.16–0.75]) and reinclusion of 1,943 patients who used RAS inhibitors or statins at baseline (0.33 [0.21–0.52]). In addition, combined use of RAS inhibitors and statins was also consistently associated with lower risks of a variety of site-specific cancers (supplemental Table, available in an online appendix at http://care.diabetesjournals.org/cgi/content/full/db09-0105/DC1). Figure 1 shows that the cumulative incidence of cancer in patients who used statins or RAS inhibitors alone or both was lower than that in those patients who were not exposed to statins and RAS inhibitors over the follow-up period.

**Prevention of renal cancer by RAS inhibitors in UNX rats.** We then explored the role of RAS activation in the development of cancers using the UNX rat model. By 10 months postoperation (Fig. 2), all of the eight untreated UNX rats (100%) developed invasive renal cell carcinoma in the remnant kidney (Fig. 2*B*). In comparison, none of the sham rats (Fig. 2*A*) or UNX rats treated with the ACEI lisinopril (Fig. 2*C*) developed renal cancer. As previously reported (27,28), untreated UNX rats also exhibited a phenotype resembling that of type 2 diabetes, characterized by insulin resistance and pancreatic β-cell deficit, whereas treatment with lisinopril significantly reduced hypertrophy of the remnant kidney (28).

**Improved lipid metabolism by RAS inhibitors in UNX rats.** We used the UNX model to examine the longitudinal effect of RAS inhibition on lipid metabolism and renal function. From 3 months onward, untreated UNX rats exhibited progressive chronic renal dysfunction, as indicated by an increased total urine protein-to-creatinine ratio (Fig. 3*A*). An elevated LDL cholesterol level was observed in the untreated UNX rats from 6 months after UNX (Fig. 3*B*). Interestingly, treatment with ACEI largely attenuated the renal dysfunction (Fig. 3*A*) and improved lipid metabolism (Fig. 3*B*) in UNX animals (all $P < 0.05$ vs. untreated UNX rats). Western blot assays of renal tissues revealed a fourfold increase in the protein expression of HMGCR in the untreated UNX rats compared with the sham animals (Fig. 4). Treatment with the ACEI lisinopril normalized the expression of HMGCR (Fig. 4).

**Normalization of IGF-1 signaling pathway by ACEI in UNX rats.** We further examined the protein expression of key molecules in the IGF-1 signaling pathway, which may be linked to carcinogenesis. Compared with sham animals, the protein expression of the growth inhibitory factor, IGFBP3, in the remnant kidney was reduced, whereas the protein expression levels of growth-promoting factors, such as Akt/PKB and PKCζ, were increased in the untreated UNX rats (Fig. 5). RAS inhibition by the ACEI nearly normalized the expression of these IGF-1 signal molecules (Fig. 5).

## DISCUSSION

This study provides evidence that the combined use of RAS inhibitors and statins may be associated with greater anticancer effects than use of either class of drugs in isolation. To explain this phenomenon, our animal data indicated that RAS blockade prevented the development of renal cell carcinoma in UNX rats via normalizing the expression of the HMGCR and IGF-1 signaling pathways.

The risk associations of statin use and cancer remain controversial. In epidemiological studies, the use of statins

TABLE 1
Clinical and biochemical characteristics of the study cohort stratified according to the occurrence of cancer during follow-up period

| | Noncancer | Cancer | *P* |
|---|---|---|---|
| *n* | 3,970 | 190 | |
| Baseline variables | | | |
| Age (years) | 54 (21) | 66 (15) | <0.0001* |
| Male sex | 1,823 (45.9%) | 98 (51.6%) | 0.1263† |
| Smoking status | | | <0.0001† |
| Ex-smoker | 541 (13.6%) | 39 (20.5%) | |
| Current smoker | 650 (16.4%) | 25.8 (49%) | |
| Alcohol drinking status | | | <0.0001† |
| Ex-drinker | 443 (11.2%) | 40 (21.%1) | |
| Current drinker | 305 (7.7%) | 17 (9.0%) | |
| BMI ($kg/m^2$) | 24.5 (4.8) | 24.4 (4.8) | 0.8547* |
| Duration of diabetes (years) | 5 (9) | 6 (9) | 0.0793* |
| Systolic blood pressure (mmHg) | 131 (25) | 135 (23) | 0.0011* |
| Diastolic blood pressure (mmHg) | 75 (13) | 75 (16) | 0.8312* |
| A1C (%) | 7.2 (2.1) | 7.3 (2.4) | 0.8346* |
| LDL cholesterol (mmol/l) | 3.20 (1.20) | 3.10 (1.40) | 0.3819* |
| HDL cholesterol (mmol/l) | 1.25 (0.45) | 1.25 (0.54) | 0.7684* |
| Triglycerides (mmol/l) | 1.28 (0.97) | 1.17 (0.74) | 0.0383* |
| Total cholesterol (mmol/l) | 5.19 (1.30) | 5.10 (1.41) | 0.2859* |
| ACR (mg/mmol) | 1.48 (5.05) | 2.71 (10.40) | <0.0001* |
| eGFR ($ml \cdot min^{-1}$ per 1.73 $m^{-2}$) | 109.2 (38.8) | 100.0 (38.1) | <0.0001* |
| Prior myocardial infarction | 18 (0.5%) | 5 (2.6%) | <0.0001† |
| Prior stroke | 107 (2.7%) | 6 (3.2%) | 0.7015† |
| Death (all-cause) | 230 (5.8%) | 93 (49.0%) | <0.0001† |
| Medications at enrollment | | | |
| Fibrates | 104 (2.6%) | 3 (1.6%) | 0.4313† |
| Use of lipid-lowering drug other than fibrates and statins | 4 (0.1%) | 0 (0.0%) | 1.0‡ |
| Antihypertensive drugs other than RAS inhibitors§ | 1,080 (27.2%) | 77 (40.5%) | <0.0001† |
| Oral antidiabetes drugs | 2,382 (60.0%) | 119 (62.6%) | 0.4328† |
| Insulin | 541 (13.6%) | 33 (17.4%) | 0.1441† |
| Medications during follow-up period‖ | | | |
| Statins only | 368 (9.3%) | 6 (3.2) | 0.0004† |
| Duration of use of statins in those who used statins only (years) | 1.71 (2.82) | 2.00 (1.40) | |
| RAS inhibitors only | 1,036 (26.1%) | 52 (27.4%) | 0.6966† |
| Duration of use of RAS inhibitors in those who used RAS inhibitors only (years) | 2.28 (3.71) | 1.49 (2.59) | |
| Both statins and RAS inhibitors | 626 (15.8%) | 17 (9.0%) | 0.00111† |
| Duration of combined use of statins and RAS inhibitors (years) | 1.77 (3.08) | 1.16 (3.22) | |
| Fibrates | 372 (9.4%) | 10 (5.3%) | 0.0555† |
| Lipid-lowering drug other than fibrates and statins | 12 (0.3%) | 1 (0.5%) | 0.4559‡ |
| Oral antidiabetes drugs | 3,284 (82.7%) | 144 (75.8%) | 0.0142† |
| Insulin | 1,312 (33.1%) | 63 (33.2%) | 0.9749† |

Data are median (interquartile range) or *n* (%). RAS inhibitors included ACEIs and angiotensin II receptor blockers. *Derived from a Wilcoxon two-sample test. †Derived from a $\chi^2$ test. ‡Derived from Fisher's exact test. §RAS inhibitors included ACEIs and angiotensin II receptor blockers. ‖From baseline (including use at baseline for all drugs except for statins and RAS inhibitors) to cancer, death or censoring dates whichever came first.

was associated with a large relative risk reduction for cancer (37). Conversely, nearly all meta-analyses of clinical trials (13,38) showed that statins have a neutral effect on incidence of cancer. However, results from meta-analyses are often inconclusive because of heterogeneity of study design, clinical profile of patient cohorts, different definitions for outcome measures, and quality of data. This statement is illustrated by the marked variations in cancer incidence ranging from 0.2 to 6.3% in these reported trials (39). Furthermore, the majority of clinical trials included in these meta-analyses were not conducted in diabetic populations.

The risk association of RAS inhibitors and cancer is also controversial. A meta-analysis of randomized trials of antihypertensive drugs failed to demonstrate a reduced odds of cancer with use of antihypertensive drugs, including RAS inhibitors (40). On the other hand, the Rotterdam Study reported that use of RAS inhibitors was associated with reduced cancer risk in ACE-DD genotype carriers, who are also known to have high levels of ACE (41). Two other studies (11,16) also showed that users of RAS inhibitors had lower risks of cancer than nonusers. Our study further showed that combined use of stains and RAS inhibitors was associated with a larger reduction in cancer risk compared with the added risk reduction associated with the use of either of the two types of drugs in isolation.

In support of these clinical observations, experimental studies indicated that RAS activation can influence carcinogenesis and tumor growth by inducing oxidative stress (42) and modulating angiogenesis, cell proliferation, immune responses, and extracellular matrix formation (12). In our experimental studies, the UNX rats developed

TABLE 2
HRs of use of RAS inhibitors and statins for cancer in type 2 diabetes

| Exposures | *n* at risk | HR (95% CI) | *P* |
|---|---|---|---|
| Main effect model 1* | | | |
| Use of RAS inhibitors | 1,770 | 0.52 (0.37–0.74) | 0.0002 |
| Use of statins | 1,056 | 0.43 (0.25–0.65) | 0.0002 |
| Main effect model 2† | | | |
| Use of RAS inhibitors | 1,770 | 0.43 (0.29–0.63) | 0.0001 |
| Use of statins | 1,056 | 0.38 (0.22–0.67) | 0.0009 |
| Multiplicative interaction model 1* | | | |
| Use of RAS inhibitors | 1,770 | 0.49 (0.34–0.71) | 0.0001 |
| Use of statins | 1,056 | 0.26 (0.10–0.65) | 0.0038 |
| Use of RAS inhibitors × use of statins | 682 | 1.98 (0.68–5.75) | 0.2117 |
| Multiplicative interaction model 2† | | | |
| Use of RAS inhibitors | 1,770 | 0.39 (0.26–0.60) | 0.0001 |
| Use of statins | 1,056 | 0.24 (0.08–0.70) | 0.0090 |
| Use of RAS inhibitors × use of statins | 682 | 1.89 (0.56–6.37) | 0.3025 |
| Additive interaction model 1* | | | |
| Use of RAS inhibitors plus nonuse of statins vs. others | 1,088 | 0.50 (0.35–0.72) | 0.0002 |
| Use of statins plus nonuse of RAS inhibitors vs. others | 374 | 0.27 (0.11–0.67) | 0.0049 |
| Use of RAS inhibitors plus use of statins vs. others | 643‡ | 0.26 (0.15–0.45) | <0.0001 |
| Additive interaction model 2† | | | |
| Use of RAS inhibitors plus nonuse of statins vs. others | 1,088 | 0.41 (0.27–0.63) | 0.0001 |
| Use of statins plus nonuse of RAS inhibitors vs. others | 374 | 0.26 (0.09–0.74) | 0.0118 |
| Use of RAS inhibitors plus use of statins vs. others | 643 | 0.20 (0.11–0.38) | <0.0001 |

Stratified Cox models on deciles of the likelihoods using statins and using RAS inhibitors during the follow-up period were used in all of the analyses. The propensity scores were calculated using logistic regression with the drug use as the dependent variable and the following variables as independent variables: age, sex, smoking status (current or ex), drinking status (current or ex), BMI, LDL cholesterol, HDL cholesterol, triglyceride, A1C, systolic blood pressure, $\log_{10}$ (ACR + 1), estimated glomerular filtration rate, duration of diabetes, peripheral arterial disease, retinopathy, sensory neuropathy, prior myocardial infarction, and prior stroke (the *c* statistics were 0.79 for use of statins and 0.80 for use of RAS inhibitors). *Adjusted for LDL cholesterol–related risk (i.e., <2.80 mmol/l plus albuminuria and ≥3.80 mmol/l), age, sex, BMI, smoking status (current plus ex), and alcohol drinking (current plus ex). RAS inhibitors included ACEIs and angiotensin II receptor blockers. †Adjusted for LDL cholesterol–related risk (i.e., <2.80 mmol/l plus albuminuria and ≥3.80 mmol/l), age, sex, BMI, smoking status, and alcohol drinking, HDL cholesterol, triglyceride, duration of diabetes, A1C, systolic blood pressure, estimated glomerular filtration rate, and medications from enrollment to cancer, death, or censoring date (oral antidiabetes drugs, insulin, and fibrates), whichever came first, and use of other antihypertensive drugs at enrollment. To avoid overfitting, the propensity score for cancer was used for all adjustments. In addition, restricted spline functions of all continuous covariates were used to calculate the propensity score to improve adjustment for nonlinear associations (the *c* statistic was 0.77). ‡39 patients who used both ACEIs/angiotensin receptor blockers and statins but at different time periods were not counted as "Use of RAS inhibitors plus use of statins."

glucose intolerance and abnormal lipid metabolism and eventually renal cancer, which were all prevented by treatment with ACEIs. This anticancer effect appears to be at least partially mediated through modulation of the HMGCR and IGF-1 signaling pathways, with the latter having complex effects on intermediary metabolism and cellular growth. The tissue activity of IGF-1 is regulated by the levels of its binding proteins as well as by the number and responsiveness of its receptors. In this regard, low levels of IGFBP3 are associated with increased risk of cancer in clinical studies. On the other hand, downstream signals of the IGF-1 pathway such as Akt/PKB and PKCζ can stimulate cell proliferation and promote cell mitosis (43). Thus, given the cancer-enhancing effects of the RAS components (12), interactions between IGF-1 signaling molecules and the tissue RAS components have been shown to stimulate the bcl-2 proto-oncogene–associated cell proliferation and to inhibit the p53 anti-oncogene–mediated cell death (44).

TABLE 3
Additive interactions of use of RAS inhibitors and statins for the risk of cancer in type 2 diabetes

| Measures of additive interaction of RAS inhibitors with statins* | Estimate (95% CI) |
|---|---|
| Model 1 | |
| RERI | 0.39 (0.09–0.69)† |
| AP | 1.57 (0.21–2.94)† |
| S | 0.66 (0.50–0.86) |
| Model 2 | |
| RERI | 0.53 (0.20–0.87)† |
| AP | 2.65 (0.38–4.91)† |
| S | 0.60 (0.46–0.78) |

*Adjusted schemes for models 1 and 2 are available in Table 2. †Statistically significant with RERI > 0, AP > 0, and S > 1 indicating additive interaction.

Cholesterol is essential for cell division and growth. In the context, IGF-1 has been shown to regulate the induction and expression of a family of genes involved in cholesterol biosynthesis (45). Thus, it is plausible that overactivation of components of the RAS, IGF-1, and HMGCR pathways may result in dysregulated growth and eventually carcinogenesis, as evidenced by *1*) reduced expression of IGFBP3, *2*) activation of the IGF-1 signaling pathway (Akt/PKB and PKCζ), and *3*) increased HMGCR expression in our UMX rat model. Of note, the interaction between the combined use of statins and RAS inhibitors was observed for multiple cancers in humans, including cancer of genitourinary organs, whereas the mechanistic exploration was made for kidney cancer in animals. In this regard, a strong association between diabetes and kidney cancer has been reported in a large cohort (46). If multiple

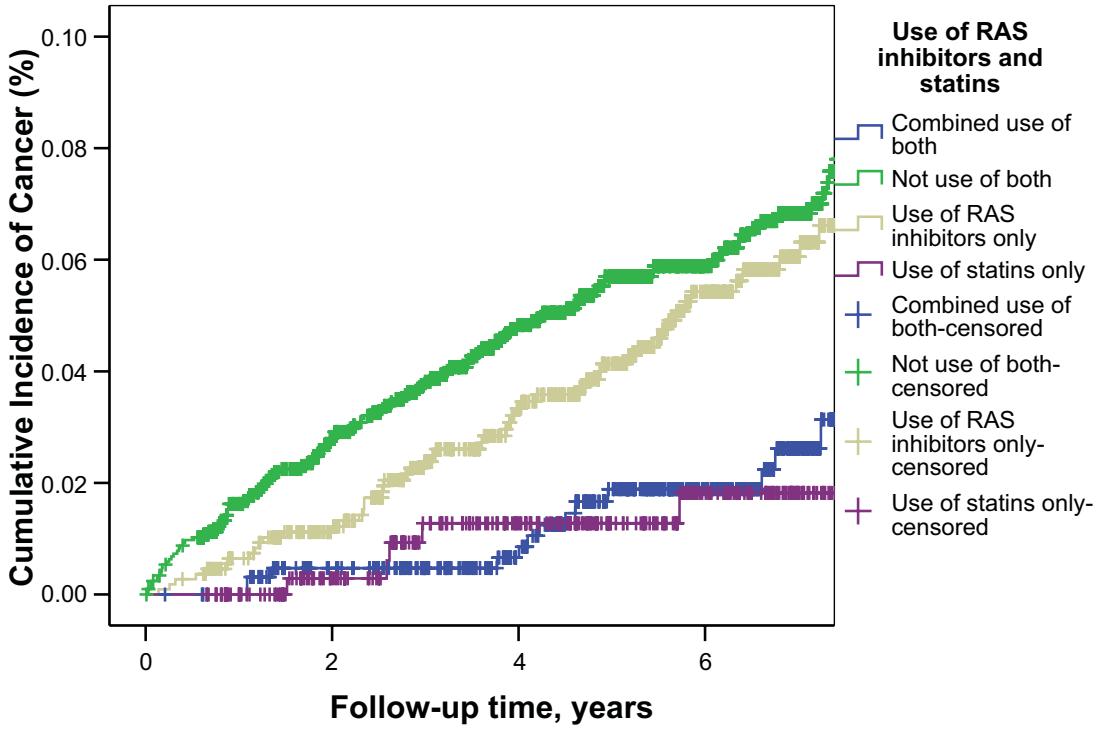


**FIG. 1. Kaplan-Meier plot showing the cumulative incidences of cancer in patients with type 2 diabetes stratified by a combination of use of statins and RAS inhibitors over the follow-period (*P* for log-rank test < 0.0001).**

cancers in type 2 diabetes share some common "pathogenesis," the IGF-1 signaling pathway is likely to play a role in development of cancer.

Our study has certain limitations. First, the study is not a clinical trial and the findings are only hypothesis generating. Second, we did not perform regular screening for cancer in this cohort because of finite resources. The use of principal discharge diagnosis to identify patients with cancer may lead to the omission of a small number of cancer events. Third, the current method of testing additive interaction does not allow us to quantify the interaction using doses of statins and RAS inhibitors. Fourth, the cohort was mainly clinic based, although the overall clinical profile of patients was comparable to that of many community-based cohorts (47). Last, the dysmetabolism observed in the animal model may not be applicable to humans, although the phenotypes exhibited by the UNX model were highly commensurate with those of type 2 diabetes.

In conclusion, we observed a synergistic effect of the combined use of RAS inhibitors and statins on reducing cancer risk in type 2 diabetes, suggesting that cross-talk of RAS and lipid metabolism may play an important role in the elevated risk of cancer in type 2 diabetes. Diabetes predisposes patients to increased risks of abnormal lipid metabolism, and elevated RAS activity is more frequent in the type 2 diabetic population than in the general population. Presumably, type 2 diabetic individuals with high LDL cholesterol or hypertension would be at risk of cancer via similar mechanisms. Thus, our findings are especially important and relevant to type 2 diabetes. Conversely, given that cross-talk between the RAS and lipid metabo-

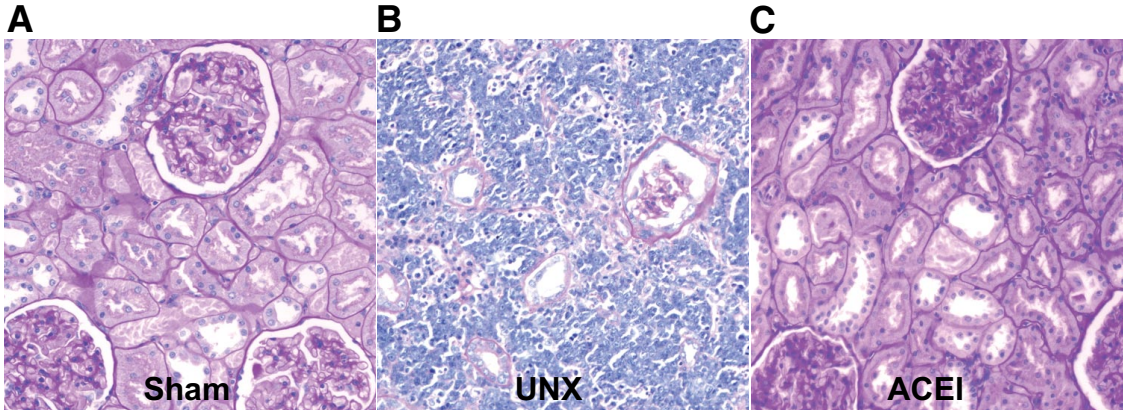


**FIG. 2. UNX-induced renal cell carcinoma in remnant kidney. Kidney tissues 10 months after the operation were obtained from sham rats (*A*), untreated UNX rats (*B*), and UNX rats treated with the ACEI lisinopril (*C*). Periodic acid Schiff stain demonstrates invasive renal cell carcinoma in remnant kidney of untreated UNX rats (*B*), but not of sham rats or UNX rats treated with the ACEI. Original magnification × 100. (A color representation of this figure is available in the online issue.)**

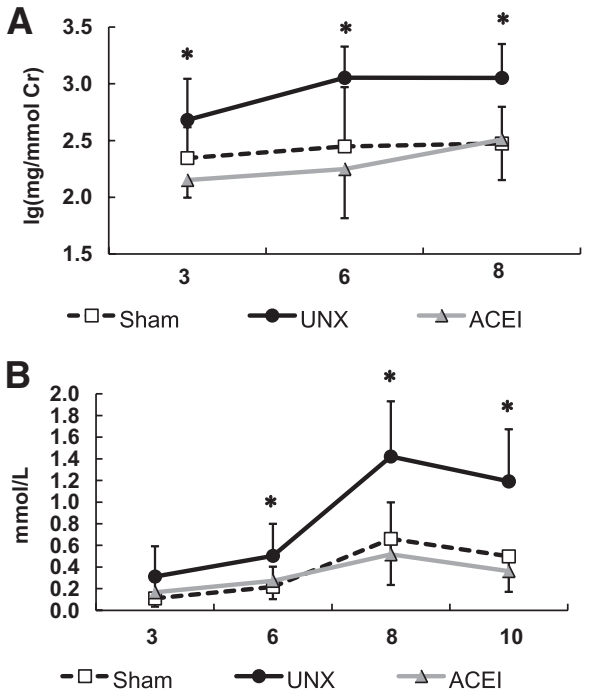


**FIG. 3. Renal dysfunction and elevated LDL cholesterol after uninephrectomy. Compared with sham rats, UNX rats progressively developed renal dysfunction, as assessed by the urine protein-to-creatinine ratio (*A*) and hyperlipidemia (*B*), as reflected by the elevated LDL cholesterol level. The proteinuria and hyperlipidemia were largely attenuated by treatment with the ACEI lisinopril. Data are means ± SD. **P* < 0.05 vs. sham and ACEI.**

lism exists in the general population for the development of atherosclerosis, whether the findings of the present study would apply to the general population warrants further investigations.


## ACKNOWLEDGMENTS

This study was partially supported by the Hong Kong Jockey Club Charities Trust [JCICM-P2–05-(CUHK)],


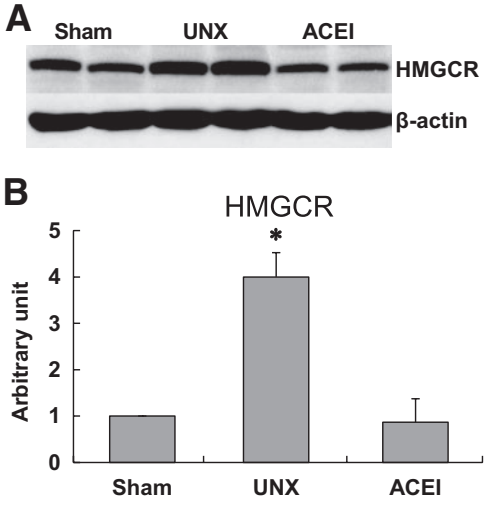


**FIG. 4. Changes in protein expression of HMGCR in renal cortex and the effects of treatment with an ACEI (lisinopril). Renal tissue specimens were obtained 10 months after the operation. Western blot assays revealed a fourfold increase of HMGCR protein expression in the remnant kidney cortex of untreated UNX rats. This overexpression of HMGCR was largely normalized by treatment with the ACEI lisinopril. **P* < 0.05 vs. sham and ACEI.**

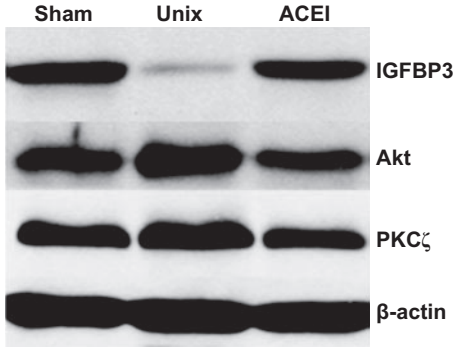


**FIG. 5. Changes in protein expression of the IGF-1 signaling pathway in renal cortex and the effects of treatment with an ACEI (lisinopril). Renal tissue specimens were obtained 10 months after the operation. Compared with sham rats, protein expression of the cancer-suppressing IGFBP3 was substantially diminished, whereas the cancer-promoting signals of Akt and PKCζ were increased in the untreated UNX rats. Treatment with an ACEI largely normalized the protein expression of these key molecules in the IGF-1 signaling pathway.**


Hong Kong Foundation for Research and Development in Diabetes, and Lioa Wun Yuk Diabetes Memorial Research Fund established under the auspices of The Chinese University of Hong Kong.

No potential conflicts of interest relevant to this article were reported.

We thank all the medical and nursing staff of the Diabetes and Endocrine Centre of the Prince of Wales Hospital for recruiting and managing the care of these patients.


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