SERUM COMPONENTS AND RISK OF CANCER - VIII

Serum Adiponectin Multimer Complexes and Liver Cancer Risk in a Large Cohort Study in Japan

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Abstract

Evidence suggests a link between adiponectin, an adipocytokine, and liver tumorigenesis. Different multimer complexes of adiponectin, with low-molecular weight (LMW), middle-molecular weight (MMW) and high-molecular weight (HMW), may have different roles. Therefore the present study was performed with the aim of assessing associations between these multimers and liver cancer development. A nested case-control study (59 liver cancer cases [mean age=63.5 years] and 334 controls [62.7 years]) was conducted as a part of the Japan Collaborative Cohort (JACC) Study recruiting healthy participants, aged 40-79 years, for the follow-up period from 1988-1990 to 1999. The end point was liver cancer occurrence/death. Serum levels of HMW, MMW and LMW adiponectin were determined at baseline using an ELISA assay. Multivariate-adjusted logistic regression analyses comparing the tertile levels of adiponectin multimers showed that the groups stratified with the highest percentage of LMW tended to have lower odds ratios (ORs) than the lowest group (OR adjusted for sex, age and area=0.54 [95%CI: 0.26-1.11] and adjusted for sex, age, area, body mass index, smoking, alcohol, coffee consumption, diabetes history and HCV-antibody positivity =0.50 [95%CI: 0.22-1.15]), albeit without statistical significance (set at p<0.05). Higher percentages of circulating LMW adiponectin may lead to a reduction of liver cancer risk and relationships with multimer composition may merit further study.

Keywords: Japan Collaborative Cohort Study - cancer - liver disease - adipocytokine

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Introduction

Obesity and its related disorders can increase the risk of various types of cancer, including liver cancer (Nair et al., 2002; Pischon et al., 2008; Watanabe et al., 2008). Although the molecular mechanisms underlying associations remain unclear, adipose tissue-derived hormones and cytokines (so-called 'adipocytokines') such as adiponectin, may be linked to tumorigenesis (Pischon et al., 2008). Adiponectin has multiple functions including anti-oxidation and anti-inflammation potential (Scherer et al., 1995; Lam and Xu, 2005). Indeed, some investigators have indicated that low circulating adiponectin levels may be associated with pathogenesis of liver cancer (Kamada et al., 2007; Nishihara et al., 2008). Furthermore, anti-fibrogenic effects of adiponectin on liver tissue might occur via suppression of proliferation and migration of activated liver stellate cells after liver damage (Kamada et al., 2003).

Adiponectin is abundantly expressed and secreted by adipose tissue, and circulates as different multimer complexes (so-called 'isoforms') such as trimers (low-molecular weight [LMW]), hexamers (middle-molecular weight [MMW]) and oligomers (high-molecular weight [HMW]) (Waki et al., 2003; Hada et al., 2007; Liu et al., 2007). Multimer complex formation of adiponectin, as a major 'quality', has become recognized as an important mechanism modulating the biological functions of this adipocytokine (Pajvani et al., 2003; Wang et al., 2006; Liu et al., 2007).

An assay system has recently been developed to measure these multimers; thus, the specific correlates of each multimer may be of particular value, rather than the assessment of total adiponectin alone. However, to date, there are only limited data available concerning the roles of these multimers in human diseases. In particular, no study has yet examined their association with human liver cancer. Therefore, the present investigation was designed to assess the relationship between the adiponectin multimer composition and risk of liver cancer in a nested case-control study in Japan.

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Subjects and Methods

Study Population and Serum Samples

This study was a part of the Japan Collaborative Cohort (JACC) Study, a nationwide multicenter collaborative and prospective study, for the Evaluation of Cancer Risk Sponsored by the Ministry of Education, Culture, Sports, Science and Technology of Japan, details of which are described elsewhere (Ohno and Tamakoshi 2001; Tamakoshi et al., 2005). The subjects were apparently healthy Japanese cohort participants (at baseline 46,465 males and 64,327 females, age 40-79 years). Of them, a total of 39,242 subjects provided blood samples. The participants were followed up from 1988-1990 until the end of 1999. The residential and survival status was annually checked by searching in the roster of residents relocating or death in each regional research center, and confirmed by death certificates for the cause and date of death under the permission from the Director-General of Prime Minister's Office. The incidence of cancer was also identified in 24 areas out of 45.

The present study on liver cancer was approved by the ethics committee of Nagoya University School of Medicine. Informed consent was obtained individually from subjects, except in certain areas in which it was provided at the group level after details had been explained to community leaders. The end point for the study was the occurrence/death of liver cancer. The current study was a nested case-control study, and the cases were selected for this study when their blood sample volumes were sufficient to fully measure all the adiponectin indices. The age-matched controls were randomly selected under the same conditions.

A lifestyle questionnaire regarding such factors as smoking, alcohol use and coffee consumption, as well as a medical history of diabetes mellitus (DM) was self-administrated at baseline. Blood samples were obtained at baseline, and these were stored at -80°C until laboratory analyses. HCV antibody (Ab; 3rd generation) was assayed by an enzyme-linked immunosorbent assay (ELISA; SRL Co. Ltd., Tokyo, Japan). The serum total, HMW, MMW and LMW adiponectin concentrations and the percentages of respective multimers were determined using an ELISA (Daiichi Co. Ltd., Tokyo, Japan) (Ebinuma et al., 2006).

Statistical analysis

The levels of total, HMW, MMW and LMW adiponectin and their percentages between cases and controls were compared using unpaired t-test. The tertile levels of these adiponectin indices among controls were evaluated by multivariate-adjusted logistic regression models to estimate odds ratio (OR) and the 95% confidence interval (CI) after adjustment for some confounders including sex, age, area, body mass index (BMI), smoking habit (never, former, current smoker), drinking habit (never, former, current drinker who consumed <2 or ≥2 Japanese drinks/day [one Japanese drink is equivalent to 23g of ethanol]), coffee consumption habit (less than 1 cup/month, 1-2 cups/month to 3-4 cups/week, almost every day), DM history and HCV-Ab positivity. In this study, model 1 was adjusted for sex, age and area, and model 2 was adjusted for sex, age, area, BMI, smoking, alcohol, coffee consumption, DM history and HCV-Ab positivity, as reported previously (Kurozawa et al., 2005; Wakai et al., 2007). All p values were two-sided, and all the analyses were performed using the Statistical Analysis System software package (SAS Institute, Cary, NC, USA).

Results

This study included 59 cases with liver cancer and 334 controls (Table 1). At baseline, the mean age±standard deviation was 63.5±7.3 years in the cases and 62.7±6.9 in the controls (with a non-significant difference in age between the two groups). Although the serum levels of total and respective multimer complexes of adiponectin were not obviously different between the cases and controls, a significantly higher percentage of MMW and a lower percentage of LMW were observed.

The multivariate-adjusted logistic regression models, adjusted for confounders (model 1: sex, age, area; model 2: sex, age, area, BMI, smoking habit, drinking habit, coffee consumption habit, DM history, HCV-Ab positivity), revealed that the groups stratified with the highest LMW adiponectin levels (in model 1: OR=0.53 [95%CI: 0.26-1.08]) and with the highest percentage of LMW (in model 1: OR=0.54 [95%CI: 0.26-1.11] and model 2: OR=0.50 [95%CI: 0.22-1.15]) tended to have lower ORs than the referent group, but this did not reach statistical significance (Table 2).

Discussion

The current study is the first to use quantitative measurements of adiponectin multimer complexes to specifically observe the contribution of the total, HMW, MMW and LMW adiponectin levels at baseline to the incidence of liver cancer. Consistently, a simple comparison test and multivariate-adjusted analyses found a high percentage of LMW might show tendency toward reduced progression of liver cancer. Although this study

Table 1. The Study Number Distribution and Serum Adiponectin Indices

	Cases	Controls
Study numbers: n (men/women)		
40-49 years	2 (1/1)	12 (4/8)
50-59 years	15 (6/9)	80 (48/32)
60-69 years	29 (18/11)	196 (121/75)
70-79 years	13 (8/5)	46 (22/24)
Total	59 (33/26)	334 (195/139)
Serum concentration of adiponectin, μg/ml		
Total	6.9±4.5	6.6±3.7
High-molecular weight	3.4±3.0	3.2±2.6
Middle-molecular weight	1.8±1.0	1.6±1.0
Low-molecular weight	1.7±1.1	1.8±0.9
Serum percentage of adiponectin multimers, %		
High-molecular weight	42.3±15.5	43.1±15.0
Middle-molecular weight	29.8±13.2	25.9±10.0*
Low-molecular weight	27.8±13.4	31.1±13.3†

Data are numbers and mean±standard deviations. Comparisons were with unpaired t-tests: *p=0.031, †p=0.083

Table 2. The Impact of Multimer Complexes of Adiponectin on Liver Cancer according to Tertiles (Odds ratios and 95%Confidence Intervals)

	T1	T2	T3	p for Trend
Serum concentration of adiponectin, μg/ml				
Total[a)]	≤4.42 (19)	4.43-7.40 (21)	≥7.41 (19)	
Model 1	1.00	1.06 (0.52-2.15)	0.90 (0.42-1.94)	0.78
Model 2	1.00	1.29 (0.57-2.92)	1.11 (0.46-2.70)	0.83
High-molecular weight				
	≤1.64 (24)	1.65-3.62 (15)	≥3.63 (20)	
Model 1	1.00	0.57 (0.28-1.17)	0.78 (0.38-1.63)	0.46
Model 2	1.00	0.71 (0.31-1.62)	1.12 (0.48-2.60)	0.84
Middle-molecular weight				
	≤1.12 (18)	1.13-1.71 (15)	≥1.72 (26)	
Model 1	1.00	0.78 (0.37-1.66)	1.29 (0.64-2.60)	0.42
Model 2	1.00	0.75 (0.31-1.84)	1.25 (0.56-2.82)	0.50
Low-molecular weight				
	≤1.34 (27)	1.35-1.99 (16)	≥2.00 (16)	
Model 1	1.00	0.54 (0.27-1.09)	0.53 (0.26-1.08)	0.066
Model 2	1.00	0.61 (0.27-1.38)	0.82 (0.37-1.84)	0.58
Serum percentage of adiponectin multimers, %				
High-molecular weight				
	≤36.5 (24)	36.6-48.9 (14)	≥49.0 (21)	
Model 1	1.00	0.56 (0.27-1.18)	0.89 (0.44-1.81)	0.71
Model 2	1.00	0.66 (0.28-1.55)	1.39 (0.61-3.14)	0.45
Middle-molecular weight				
	≤21.0 (15)	21.1-29.0 (17)	≥29.1 (27)	
Model 1	1.00	1.13 (0.53-2.42)	1.70 (0.83-3.45)	0.13
Model 2	1.00	0.74 (0.30-1.78)	1.03 (0.45-2.36)	0.82
Low-molecular weight				
	≤24.8 (27)	24.9-36.8 (17)	≥36.9 (15)	
Model 1	1.00	0.58 (0.29-1.15)	0.54 (0.26-1.11)	0.079
Model 2	1.00	0.52 (0.23-1.13)	0.50 (0.22-1.15)	0.084

The tertile level ranges and the study numbers for liver cancer cases (n) are shown for the respective adiponectin indices. The indices were based on the distribution of concentrations among the controls, numbering 111, 111 and 112 in tertile categories T1, T2 and T3, respectively; Model 1, adjusted for sex, age, area; Model 2, adjusted for sex, age, area, body mass index, smoking, alcohol, coffee consumption, diabetes history, HCV-antibody positivity

followed a relatively modest number of cancer cases, which could have limited the statistical power (this is a study limitation), it is interesting to note that the risk ratio reduced by approximately half at the higher level of the percentage of LMW adiponectin in comparison to the lowest level of the percentage (as a referent). In addition, while the total adiponectin levels did not show any relevance to liver cancer in this analysis, the measurements of multimer complexes may address the clinical implication of adiponectin on liver cancer or lead to a deeper understanding of the pathophysiology.

Once the multimer complexes appear in blood, they do not interconvert with each other; thus, their secretion from adipocytes reflects the blood levels of each complex (Schraw et al., 2008). HMW adiponectin has been described as a predominant active and possible protective multimer because of decreased HMW adiponectin in subjects with obesity and multiple cardiovascular risk factors (Lara-Castro et al., 2006; Inoue et al., 2007). Yet, there has not always been a consensus regarding such opinions. In fact, by contrast, it has been known that HMW adiponectin exerts pro- and LMW adiponectin exerts anti-inflammatory effects (Tsao et al., 2003; Wulster-Radcliffe et al., 2004; Abke et al., 2006; Neumeier et al., 2006; Rovin and Song 2006; Schober et al., 2007): for instance, HMW adiponectin enhances the release of interleukin-6 from human monocytes, whereas LMW adiponectin inhibit the release of this pro-inflammatory cytokine (Schober et al., 2007). Furthermore, a recent report showed that HMW adiponectin may exert less bioactivity in the metabolic traits among certain ethnic groups, such as African-Americans (Lara-Castro et al., 2008). Therefore, it is possible that the relevance of multimer complexes differs between a variety of disorders and populations. In this context, the current study results could in part suggest the beneficial effects of LMW adiponectin on the progression of liver cancer.

The study limitations warrant mention. The serum biomarkers were analyzed using a single sample collected at baseline, and the concentrations of the adiponectin indices may have changed somewhat over time. The combined use of the incidence and death due to liver cancer as the study end point can be also a considered point.

In summary, higher percentages of serum LMW adiponectin may substantially act in the suppression of liver cancer, although there was no marked significance at a statistical level in this cohort study. The relationship between the serum adiponectin multimer complexes and liver cancer is considered to merit further study.

Member List of the JACC Study Group

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