

not large enough to observe a positive association, and the possibility of an adverse effect on the colon cancer from a greater intake than in our highest quartiles of processed meat cannot be excluded. The different results between pork and processed meat might be partly attributable to relatively low level of processed meat intake among the Japanese. In this study, the results did not support a hypothesis that higher processed meat or other meat intake increases the risk of rectal cancer with these levels. Third, although we measured and adjusted for possible confounding variables to the extent possible, the possibility of confounding by unmeasured variables cannot be totally disregarded. Also, it is possible that some of the significant findings may be due to chance.

In conclusion, in this large-scale, population-based prospective cohort study among middle-aged Japanese men and women, whose consumption of red meat was considered moderate by Western standards, we found that higher consumption of red meat was associated with an increased risk of colon cancer among women, as was higher consumption of total meat among men. The positive associations for subsite-specific colon cancers appeared to differ by gender. The Japanese may be particularly susceptible to the adverse effects of red meat intake in the development of colon cancers.

AUTHOR DISCLOSURES

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Should Elderly Patients Undergo Additional Surgery After Non-Curative Endoscopic Resection for Early Gastric Cancer? Long-Term Comparative Outcomes

Chika Kusano, MD, PhD¹, Motoki Iwasaki, MD, PhD², Tonya Kaltentbach, MD, PhD³, Abby Conlin, MD⁴, Ichiro Oda, MD⁵ and Takuji Gotoda, MD, PhD¹

- OBJECTIVES:** Endoscopic resection (ER) including endoscopic submucosal dissection has been widely accepted for treatment of early gastric cancer (EGC) in Japan. Additional surgery is recommended when ER is non-curative histologically. Many elderly patients, however, do not undergo radical surgery due to comorbid disease or limited life expectancy. The aim of this study is to assess the survival outcomes of radical surgery compared with observation only in elderly patients after non-curative ER.
- METHODS:** We reviewed existing data of all elderly patients (older than 75 years) who had undergone ER for EGC at the National Cancer Center Hospital between January 1999 and December 2005. We compared the overall and disease-free survival rates between three patients groups: curative ER, non-curative ER with additional surgery, and non-curative ER without additional surgery.
- RESULTS:** In total, 428 patients underwent ER; 308 (72%) curative ER and 120 (28%) non-curative ER. Of the 120 non-curative ER patients, 38 patients (31.7%) underwent additional surgery and 82 patients (68.3%) were followed without surgery. There was no significant difference in American Society of Anesthesiologist score between three groups. Patients who did not undergo surgery tended to be older. Overall 5-year survival rates in the curative ER, non-curative ER with surgery, and non-curative ER without surgery were 85, 92, and 63%, respectively. There was no significant difference in overall and disease-free survival between patients in the curative ER and non-curative ER with surgery groups. On the contrary, a significant difference in overall and disease-free survival was evident between the curative ER and non-curative ER without surgery groups (hazard ratio (95% confidence interval): 1.89 (1.08–3.28), 2.30 (1.35–3.94)).
- CONCLUSIONS:** In our elderly patient cohort, additional surgery following non-curative ER improved overall and disease-free survival compared with non-surgical observation only. Thus, surgery should be considered following non-curative ER in EGC patients >75 years of age.

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INTRODUCTION

Life expectancy in elderly patients has increased dramatically worldwide (1,2). Although surgical techniques and preoperative management have improved minimally invasive curative treatment is preferable for the elderly, particularly for early stage cancer (EGC).

Endoscopic resection (ER) has been accepted as standard treatment for EGCs that meet guideline or expanded criteria (3,4), which have a low risk of lymph node metastasis. Following ER, meticulous

pathological evaluation of the resected specimen is used to stratify patient management. Patients with lesions that meet the guideline or expanded criteria are followed closely, whereas those who have had a non-curative ER are considered for additional surgery.

Gastrectomy is associated with high surgical risk for the general population. Partial or total gastrectomy is also associated with short and long-term morbidity, and mortality (5,6). Furthermore, the majority of elderly patients who are 75 years or older

¹Gastroenterology and Hepatology Division, National Center for Global Health and Medicine, Tokyo, Japan; ²Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan; ³Endoscopy, GI Section, Veterans Affairs Palo Alto Health Care System and Stanford University School of Medicine, Palo Alto, California, USA; ⁴Department of Gastroenterology, Manchester Royal Infirmary, Manchester, UK; ⁵Endoscopy Division, National Cancer Center Hospital, Tokyo, Japan. **Correspondence:** Chika Kusano, MD, PhD, Gastroenterology and Hepatology Division, National Center for Global Health and Medicine, 1-2-1 Toyama, Shinjyuku-ku, Tokyo 162-8655, Japan. E-mail: ckusano2007@yahoo.co.jp
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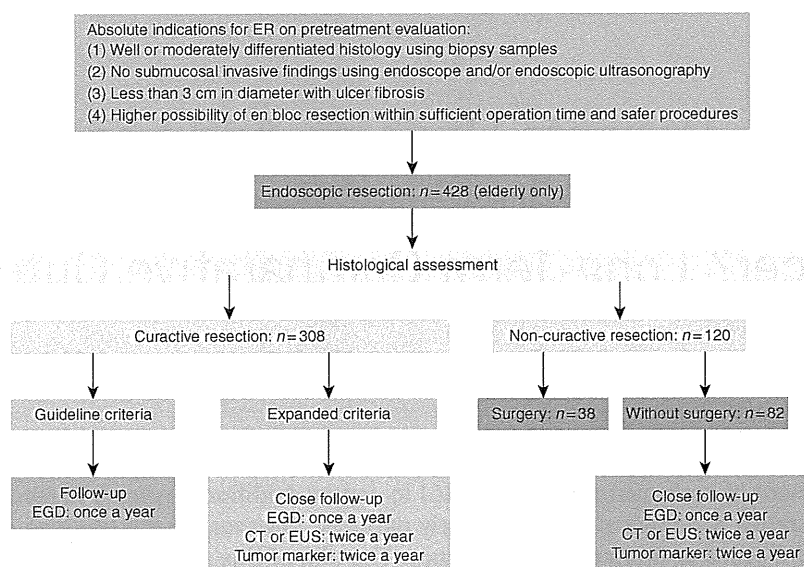


Figure 1. Flowchart of critical procedure. CT, computed tomography; EGD, endogastroduodenoscopy; ER, endoscopic resection; EUS, endoscopic ultrasonography.

have multiple diseases and functional disorders influencing daily life (7,8). In this study, we describe the long-term outcomes of ER for EGC in patients aged 75 years or older. We primarily aim to determine whether lesions beyond the guideline or expanded criteria in this elderly cohort can be treated adequately with ER alone.

METHODS

Study design

We reviewed existing data on all patients who had undergone ER for EGC at the National Cancer Center Hospital, Tokyo, between January 1999 and December 2005. Patients whose lesions did not meet criteria for ER following preoperative diagnosis were excluded. We defined elderly patients as 75 years or older (7). Elderly patients were divided into three groups: curative ER, non-curative ER with additional radical surgery, and non-curative ER without surgery. We used the American Society of Anesthesiologist (ASA) score and Charlson Index (9) as a measurement of patients overall health status, and surgical risk. All patients provided written informed consent.

Method

Starting in 1999, our institution has routinely followed a standard protocol for the ER of EGC.

Indication for ER

Indication criteria for ER—"differentiated histology," "macroscopic absence of submucosal invasive findings using endoscope and/or endoscopic ultrasonography," "lesion size- <3 cm in diameter with ulcer fibrosis," and "high probability of safe en bloc resection with short procedure duration." Patients deemed unfit for open surgery due to their general condition were also judged to be poor candidates for ER (Figure 1).

Historical assessment

Resection specimens were classified according to the Japanese Classification for Gastric Carcinoma (10). In this study, ER was declared curative when the specimen showed en bloc resection with margins free of cancer and if applicable, met the expanded criteria: (i) intramucosal cancer, differentiated type, no lymphatic or/and venous invasion, and no ulceration, irrespective of tumor size; (ii) intramucosal cancer, differentiated type, no angiolymphatic invasion, and tumor <3 cm in size, irrespective of ulceration findings; (iii) minimally invasive submucosal cancer (invasion depth $\leq 500 \mu\text{m}$, sm1), differentiated type, no lymphatic or/and venous invasion, and tumor <3 cm in size.

Post ER management

All patients were followed according to our standard protocol (Figure 1). Surveillance upper endoscopy was performed annually. Curative cases with expanded criteria also underwent abdominal computed tomography or endoscopic ultrasonography and tumor-marker studies (carcinoembryonic antigen, CA19-9) every 6 months to exclude lymph node or distant metastasis. Patients who underwent non-curative ER and were deemed fit for surgery were referred and consented for radical resection and lymph node dissection. Patients with the non-curative ER without surgery due to physician judgment or strong patient refusal were followed up by the same protocol as patients with curative resection with expanded criteria.

Statistical analysis

Differences in patient characteristics between the three groups were examined by χ^2 test. Survival curves were calculated using the Kaplan-Meier method. To compare overall and disease-free survival among the treatment status, Cox proportional-hazards model was performed to estimate hazard ratio (HR) and 95% confidence interval (CI). The following covariates were included

in the multivariable analyses: age, sex, ASA score, past history of cancer (stratified by cancer stage), and comorbid illnesses. We also compare the overall and disease-free survival in the multivariable analyses included age, sex, and Charlson Index. All *P* values reported are two-sided, and significance level was set at *P* < 0.05. All statistical analyses were performed with the SAS software version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

Patient characteristics

A total of 2,012 cases (2,399 lesions) of EGC were treated endoscopically at the National Cancer Center Hospital between January 1999 and December 2005. Of these, 1,947 cases (2,331 lesions) met the indication for ER following preoperative diagnosis. In all, 428 (519 lesions) of the 1,947 cases were elderly (75 years or older). Of these cases in elderly patients, 26 lesions were treated by endoscopic mucosal resection and 493 lesions were treated by endoscopic submucosal dissection. A total of 308 elderly patients (72%, 308/428) had a curative ER and 120 patients (28%, 120/428) had a non-curative ER. Of the 120 patients with non-curative ER, 38 patients (31.7%, 38/120) underwent radical surgery and 82 patients (68.3%, 82/120) were followed without surgery.

Patient characteristics are summarized in **Table 1**. ASA score of all patients except nine was 2. In all, 312 patients (72.9%, 312/428) were Charlson Index 2, 65 patients (15.2%, 106/428) were 3, 41 patients (9.6%, 41/428) were 4, and 10 patients were over 5 (2.3%, 10/428). There was no significant difference in ASA score and Charlson Index between three groups (ASA score, *P* = 0.17; Charlson Index, *P* = 0.33). There was a significant difference in age and the prevalence of cardiovascular disease. Patients who did not undergo surgery tended to be older.

Reasons for not undergoing surgery in the remaining 82 patients included patients' choice (*n* = 29), physicians' judgment (*n* = 45) (including 10 very elderly (mean age 84 years), one with chronic renal dysfunction, one with ventilatory impairment and one with aneurysm of the thoracic aorta, concomitant cancer in other organs (*n* = 7)) and unknown (*n* = 8).

Survival

The median follow-up period in the curative ER, non-curative ER with surgery, and non-curative ER without surgery was 40.6, 43.1, and 38.1 months, respectively. Overall 5-year survival in each group was 84, 95, and 63%, respectively (**Table 2**). Using ASA score, age, sex, clinical stage of cancer in past history, and past history of diseases, there was no significant difference in overall and disease-free survival between the patients with curative ER (*n* = 308) and non-curative ER with surgery (*n* = 38). On the contrary, a significant difference in overall and disease-free survival was evident between the patients with curative ER (*n* = 308) and non-curative ER without surgery (*n* = 82) (HR (95% CI): 1.89 (1.08–3.28), 2.30 (1.35–3.94); **Table 2**, **Figure 2**). The multivariable analysis using Charlson Index, age, and sex shows a statistical difference in overall and disease-free survival between the patients with curative ER and non-curative ER without surgery

Table 1. Patient characteristics

	Curative resection	Non-curative resection with surgery	Non-curative resection without surgery
Number of patients (%)	308 (72.0)	38 (8.9)	82 (19.2)
Age, mean (s.d.)	78.8 (3.3)	76.9 (2.3)	80.1 (3.9)
Gender ratio, men: women	228:80	32:6	67:15
Concomitant disease (%)			
Cancer	59 (19.2)	3 (7.9)	13 (15.9)
Cardiovascular diseases	48 (15.6)	16 (42.1)	11 (13.4)
Diabetes	29 (9.4)	6 (15.8)	7 (8.5)
Respiratory diseases	6 (1.9)	1 (2.6)	3 (3.7)
Other diseases	15 (4.9)	2 (5.3)	6 (7.3)
ASA score (%)			
2	304 (100)	37 (100)	78 (98.7)
3	0	0	1
Missing information	4	1	3
Charlson Index			
2	232 (75.3)	25 (65.8)	55 (67.1)
3	43 (14.0)	8 (21.1)	14 (17.1)
4	25 (8.1)	4 (10.5)	12 (14.6)
5+	8 (2.6)	1 (2.6)	1 (1.2)
ASA, American Society of Anesthesiologist.			

(HR (95% CI): overall survival, 2.35 (1.36–4.05); disease-free survival, 2.76 (1.64–4.67)).

In total, 59 patients (13.8%, 54/428) died during this study period. The majority (55.9%, *n* = 33/59) of deaths occurred in the curative ER group followed by the non-curative ER without surgery group (40.7%, *n* = 24/59). Only two (3.4%) deaths occurred in the group who had non-curative ER with surgery. Of the 428 patients, 1.2% (*n* = 5) died as a result of gastric cancer and 12.6% (*n* = 59/432) died from another causes (**Table 2**). Of the five patients who died of gastric cancer, one patient died from metachronous advanced gastric cancer following curative ER of the index lesion. Four patients in the non-curative ER without surgery died from lymph node metastasis or distant metastasis. There were no deaths from cancer recurrence in the non-curative ER with surgery.

Survival according to the risk of lymph node metastasis

We divided non-curative ER groups into two groups according to the risk of lymph node metastasis: A—high risk (“positive lymphatic or/and venous invasion” or “submucosal deep (sm2) invasion”) and B—low risk (other reasons except high risk of lymph node metastasis such as intramucosal cancer > 30 mm in size with ulcer findings and minute submucosal cancer

Table 2. Hazard ratio (HR) and 95% confidence intervals (CIs) of overall survival according to curability

	Number of deaths (death from gastric cancer)	Five-year survival rate (%)	Crude		Multivariable adjusted ^a	
			HR	95% CI	HR	95% CI
Curative ER	33 (1)	84	1.00		1.00	
Non-curative ER with surgery	2 (0)	95	0.52	0.13–2.17	0.70	0.16–2.98
Non-curative ER without surgery	24 (4)	63	2.62	1.54–4.46	1.89	1.08–3.28

ASA, American Society of Anesthesiologist; ER, endoscopic resection.

^aAdjusted for age, sex, ASA score, clinical stage of cancer in past history, and past history of diseases (cardiovascular diseases, diabetes mellitus, respiratory diseases, and others).

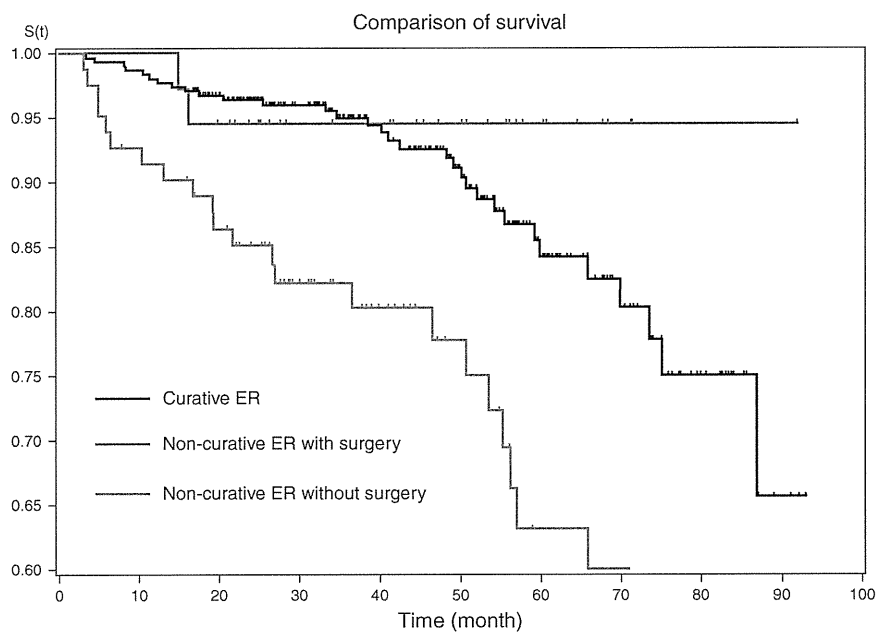


Figure 2. Survival for elderly patients (overall survival). ER, endoscopic resection.

(sm1) >30 mm in size). Among the non-curative ER patients, 29 of the 67 high-risk patients (43.3%) underwent additional surgery compared with only 9 patients of the 53 low-risk patients (17.0%). **Table 3** shows overall survival according to the risk of lymph node metastasis using ASA score, age, sex, clinical stage of cancer in past history, and past history of diseases. Overall 5-year survival rate in non-curative ER-A without surgery group was lowest (52%). There were significant difference in overall and disease-free survival between the patients with curative ER ($n=308$) and non-curative ER-A without surgery group (HR (95% CI): 3.31 (1.67–6.58), 4.26 (2.20–3.94); **Table 3**). In the multivariable analysis using Charlson Index, age, and sex, a statistical significance was evident in overall and disease-free survival between the patients with curative ER and non-curative ER-A without surgery (HR (95% CI): overall survival, 4.15 (2.18–7.89); disease-free survival, 5.30 (2.85–9.84)).

DISCUSSION

Surgery continues to be the mainstay of treatment for gastric cancer—with a reported high resection rate (96%) and a low surgical complication rate (8%) even in elderly patients (11). However, 5-year survival after surgery in elderly patients varies among institutions, and is reported to be 69–74% for EGC. This is compared with 5-year survival rates of >90% in young and middle-aged patients (12). Age-related disease, in fact, is the main etiology of the relatively low survival in elderly patients. Thus, less invasive surgical treatment is desirable in the elderly, and ER is attractive in this respect.

ER targets EGC lesions that have a negligible likelihood of lymph node metastasis, estimated at <1% for intramucosal cancer and <3% for submucosal invasive cancer (4). Several recent studies have reported that endoscopic submucosal dissection can be carried out on larger lesions resulting in a high rate of cancer-free

Table 3. Hazard ratio (HR) and 95% confidence intervals (CIs) of overall survival according to the risk of lymph node metastasis

	Number of subject	Number of deaths (death from gastric cancer)	Five-year survival rate (%)	Crude		Multivariable adjusted ^a	
				HR	95% CI	HR	95% CI
Curative ER	308	33	84	1.00		1.00	
Non-curative ER-A with surgery	29	1 (0)	96	0.36	0.05–2.66	0.54	0.07–4.07
Non-curative ER-B with surgery	9	1 (0)	89	0.96	0.13–7.01	1.09	0.15–8.14
Non-curative ER-A without surgery	38	14 (3)	52	4.72	2.52–8.85	3.31	1.67–6.58
Non-curative ER-B without surgery	44	10 (1)	71	1.55	0.75–3.22	1.17	0.56–2.47

ASA, American Society of Anesthesiologist; ER, endoscopic resection.

^aAdjusted for age, sex, ASA score, clinical stage of cancer in past history, and past history of diseases (cardiovascular diseases, diabetes mellitus, respiratory diseases, and others).

margin (13,14). Long-term survival of EGC patients undergoing ER with expanded criteria has been equal to those undergoing ER with original guidelines (15). Expanded criteria for ER of larger tumors may benefit elderly patients with EGC (16).

As a general rule, additional surgery should be recommended for patients when curative ER is not achieved (17), as EGC surgical outcomes are known to be excellent (11). Our study provides long-term survival data of EGC in an elderly cohort. We demonstrate the efficacy of curative ER for EGC, showing a similar 5-year survival rate among elderly patients with curative ER and non-curative ER with surgery. We found that when curative ER was not achieved, elderly patients appeared to benefit from subsequent surgical gastrectomy. Furthermore, patients who had a non-curative ER without surgery and were established to have a high risk of lymph node metastasis had the lowest overall 5-year survival rate of 52%.

It was reported that lymphovascular involvement and massive submucosal penetration had a significant association with lymph node metastasis in EGC (18). From our data, there were significant difference in overall and disease-free survival between the patients with curative ER and non-curative ER-A without surgery group. Lymphovascular involvement or massive submucosal penetration was more frequent in surgical patients than in non-surgical patients. It is likely that the physician suggested additional surgery to these patients with high risk of lymph node metastasis. Considering the patient's age and the risk of lymph node metastasis in this recommendation.

Notably, the patients with the non-curative ER without surgery did not undergo additional surgery primarily due to subjective measures. Thus, although the treating physician routinely discussed and recommended radical surgery to all patients with non-curative ER, individual factors such as comorbid disease, reason for non-curative ER, age, and patient preference ultimately influenced treatment decisions. These conditions are subjective and cannot be expressed numerically, and are an inherent limitation of our retrospective study.

In conclusion, following non-curative ER for EGC, especially with lymphovascular involvement or massive submucosal penetration, additional surgery is recommended in elderly patients.

CONFLICT OF INTEREST

Guarantor of the article: Chika Kusano, MD, PhD.

Specific author contributions: Conceptualization, data analysis, and script preparation: Chika Kusano and Motoki Iwasaki; endoscopic diagnosis and treatment: Takuji Gotoda and Ichiro Oda; data collection: Chika Kusano, Ichiro Oda, and Takuji Gotoda; critical reviewer of the paper: Ichiro Oda, Takuji Gotoda, Tonya Kaltenbach, and Abby Conlin. All authors have read and approved the submitted version of the paper.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Endoscopic resection (ER) has been accepted as standard treatment for early gastric cancers, which have a low risk of lymph node metastasis.
- ✓ Additional surgery with lymph node dissection should be recommended for patients when curative ER is not achieved.
- ✓ Deciding whether or not to pursue gastric surgery or not is particularly complex in elderly patients who often have comorbidities and limited life expectancy.

WHAT IS NEW HERE

- ✓ A significant difference in overall and disease-free survival was evident between the patients with curative endoscopic resection (ER) and non-curative ER without surgery (hazard ratio (95% confidence interval): 1.89 (1.08–3.28), 2.30 (1.35–3.94)).
- ✓ Overall and disease-free survival of non-curative ER with "positive lymphatic or/and venous invasion" or "submucosal deep (sm2) invasion" are lowest.
- ✓ After non-curative ER for early gastric cancer, especially with lymphovascular involvement or massive submucosal penetration in historical findings, additional surgery is necessarily even in elderly patients.

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Research Article

Plasma Isoflavones and the Risk of Lung Cancer in Women: A Nested Case–Control Study in Japan

Taichi Shimazu, Manami Inoue, Shizuka Sasazuki, Motoki Iwasaki, Norie Sawada, Taiki Yamaji, and Shoichiro Tsugane; for the JPHC Study Group

Abstract

Background: Although several epidemiologic studies have found that isoflavone intake assessed by questionnaire is associated with a decreased risk of lung cancer, no prospective study has investigated this association using blood concentrations of isoflavones.

Methods: We conducted a nested case–control study within a population-based prospective cohort study. A total of 24,127 women aged 40 to 69 years who returned the baseline questionnaire and provided blood samples were observed from 1990 through 2006. During a median follow-up period of 13.5 years, 126 newly diagnosed lung cancer cases were identified. For each case, we selected two controls matched for age, area, smoking status, and condition of blood draw. A conditional logistic regression model was used to estimate the odds ratios (ORs) and 95% CIs of lung cancer in relation to plasma concentrations of genistein, daidzein, glycitein, equol, and total isoflavones.

Results: After exclusion of 20 lung cancer cases diagnosed in the first 3 years after blood collection, an inverse association was found between plasma genistein concentration and lung cancer risk. The multivariate-adjusted OR (95% CI) of lung cancer in the highest quintile of plasma genistein concentration as compared with that in the lowest quintile was 0.31 (0.12, 0.86; *P* for trend = 0.085). Other isoflavones and total isoflavones were not associated with a significant decrease in the risk of lung cancer.

Conclusion: Plasma genistein concentration was inversely associated with lung cancer risk in Japanese women.

Impact: Our data support the previously observed association between isoflavone intake and lung cancer risk. *Cancer Epidemiol Biomarkers Prev*; 20(3); 419–27. ©2011 AACR.

Introduction

Isoflavones, including genistein, daidzein, and glycitein, are found mainly in soy and soy products in Asian diets. They are similar in structure to the human female hormone 17- β estradiol. They are also similar in function, as they have a high affinity for the beta-estrogen receptor (1) and act as estrogen agonists and antagonists

(2). Therefore, it has been hypothesized that isoflavones protect against the development of cancers related to sex hormones. Indeed, epidemiologic studies have shown an inverse association between isoflavones and the risks of breast (3–5) and prostate cancers (6–8).

In addition to these cancers, it has been suggested that estrogen has a role in lung carcinogenesis (9). Estrogen receptors are expressed in healthy lung tissue and in lung tumors (10), and estrogen induces proliferation of non-small-cell lung cancer (NSCLC) cells (11). Furthermore, randomized controlled trials have indicated that hormone replacement therapy which includes estrogens may increase lung cancer risk in women (12, 13). Thus, isoflavones may be related to the risk of lung cancer, in addition to other hormone-related cancers.

Although several *in vitro* and *in vivo* studies have shown a protective effect of genistein on lung carcinogenesis (14–16), epidemiologic studies have produced conflicting results regarding the association between lung cancer risk and isoflavone intake assessed by food frequency questionnaire (FFQ; ref. 17–20). Notably, 2 recent prospective studies in Asia observed an inverse association in never smokers (19, 20). Epidemiologic

Authors' Affiliation: Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan

Note: Study group members are listed in the Appendix.

Authors' Contributions: T. Shimazu, M. Inoue, and S. Tsugane designed research; T. Shimazu, M. Inoue, S. Sasazuki, M. Iwasaki, N. Sawada, T. Yamaji, and S. Tsugane conducted research; T. Shimazu analyzed data and wrote the paper; T. Shimazu had primary responsibility for final content. All authors read and approved the final manuscript.

Corresponding Author: Taichi Shimazu, Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, 104-0045 Japan. Phone: 81-3-3542-2511 (ext 3389); Fax: 81-3-3547-8578. E-mail: tshimazu@ncc.go.jp

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studies using blood concentrations of isoflavones might clarify the association with lung cancer risk, because the concentration of isoflavone in blood reflects individual differences in absorption and metabolism, in which intestinal microflora have an important role (21). In particular, due most likely to differences in intestinal bacteria, only 30% to 50% of adults have the capacity to metabolize daidzein into equol, which is known to have stronger estrogenic activity than daidzein (22).

Here, in a nested case-control study within a large-scale, population-based, prospective study, we investigated the association between plasma isoflavone concentration and lung cancer risk among a population of Japanese women that varied substantially in isoflavone intake (3) and had a high prevalence of never smokers (23).

Materials and Methods

Study population

The Japan Public Health Center-based Prospective Study was launched in 1990–1994. The study population was defined as all Japanese inhabitants who had registered their address in administrative districts (city, town, or village) supervised by the 11 public health center (PHC) and were aged 40 to 69 years at the start of the baseline survey (24). Study participants were informed of the objectives and methods of the study in writing, and those who responded to the survey questionnaire and donated blood were regarded as having given informed consent to participate in the study. In addition, participants were notified that they could withdraw from the study at any time. Our study protocol follows the current ethical guidelines for epidemiological research in Japan (25). The study protocol was approved by the Institutional Review Board of the National Cancer Center, Japan.

For the present analysis we excluded one PHC area because data on cancer incidence were not available. After exclusion of ineligible participants ($n = 144$), we identified 67,522 women as the cohort.

Questionnaire survey

We distributed a baseline self-administered questionnaire survey on various health habits, including personal medical history, menstrual and reproductive history, anthropometric factors, smoking history, and other lifestyle factors in 1990 for Cohort I and in 1993 to 1994 for Cohort II. Women who reported first-degree relatives with lung cancer were considered to have a family history of lung cancer. Never smoking status was determined by answers to the question "Have you ever smoked cigarettes?" in Cohort I and by answers to the question "Are you currently smoking cigarettes?" and information on past history of smoking in Cohort II. Questions regarding age at initiation of smoking and average number of cigarettes smoked per day were also included. Information on passive smoking at the workplace was collected

using a question with 4 frequency categories: almost never, 1–3 days/month, 1–4 days/week, and almost daily. We defined women who drank alcoholic beverages less than 1 day/month as nondrinkers. Women who reported past or current use of female hormone drugs were classified as past or current exogenous female hormone users. We had no information on the type, duration, or dosage of such use.

The questionnaire survey also included validated FFQs that asked about average intake during the previous month of 44 food items (for Cohort I) or 52 food items (for Cohort II). The questionnaires had 6 frequency categories for beverages, ranging from 'rarely' to '5 glasses per day', and 4 (Cohort I) or 5 (Cohort II) categories for other items, ranging from 'never' or 'rarely' to 'almost daily'. The intakes of total energy, vegetables, fruit, and fish were calculated from these responses (26, 27), and portion sizes were estimated using data from a validation study (28).

Each questionnaire included 3 food items that contained genistein. In Cohort I, the percentages of women reporting 'almost daily' consumption of (i) miso soup, (ii) soybeans, tofu, deep-fried tofu, and natto (fermented soybeans), and (iii) vegetables other than yellow and green vegetables (e.g., Chinese cabbage, radish, tomato, and cucumber) were 79.4%, 52.2%, and 46.7%, respectively. In Cohort II, miso soup, tofu, and natto were consumed almost daily by 63.2%, 32.5%, and 9.5% of women, respectively.

A total of 55,842 women responded to the questionnaire, yielding a response rate of 83%. We then excluded 585 participants with incomplete information on smoking status and 1,525 participants who had received a diagnosis of cancer before the baseline questionnaire survey. Ultimately, a total of 53,732 women were eligible.

Blood collection

Participants voluntarily provided 10 mL of blood during health checkups in 1990–1995. Blood samples were divided into plasma and buffy layers and preserved at -80°C until analysis. Among the eligible participants, a total of 24,127 women (96.7% of participants in health checkups) donated blood.

Follow-up

We followed study participants until December 31, 2006. Participants who died or moved to other municipalities were identified annually through residential registers in the respective PHC areas. Cause of death was confirmed using mortality data from the Ministry of Health, Labour and Welfare. Among the study participants ($n = 24,127$), 1,160 (4.8%) died, 1,559 (6.5%) moved away, and 51 (0.2%) were lost to follow-up during the study period.

Selection of cases and controls

We determined lung cancer incidence by using voluntary reports from local major hospitals in the study areas

and data linkage with population-based cancer registries, after obtaining permission. We used death certificate information as a supplementary information source. In our cancer registry system, the proportion of cases for which information was obtained only from death certificates was 5.1% during the study period. During the time from blood collection to the end of the study period, we identified 126 newly diagnosed lung cancer cases.

The site of origin and histologic type were coded using the International Classification of Diseases for Oncology, Third Edition (C34.0-C34.9; ref. 29). Diagnosis of lung cancer was confirmed by histologic or cytologic examination in 89% of cases ($n = 112$), and was based on clinical findings or unspecified evidence in the remaining 11%. Histologic type was classified as adenocarcinoma ($n = 94$; 75%), squamous cell carcinoma ($n = 6$), large cell carcinoma ($n = 4$), small cell carcinoma ($n = 3$), or other histologic types ($n = 5$), according to the World Health Organization histological classification of lung tumors (30).

For each case, 2 controls were selected at random from participants with no history of lung cancer when the case was diagnosed. Controls were matched for each case by age (within 3 years), PHC area, area (city, or town and village), date on which blood was collected (within 60 days), time of day of blood collection (within 3 hours), duration of fasting at blood collection (within 3 hours), and smoking status (never, past, and current).

Laboratory assays

Plasma concentrations of isoflavones (i.e., genistein, daidzein, glycitein, and equol) were analyzed using triple-quadrupole tandem liquid chromatography–mass spectrometry (31). Beta-glucuronidase/sulfatase was added to 0.1 mL of plasma. The aglycones of the isoflavones and their metabolites were recovered by diethyl ether extraction. The diethyl ether extract of the sample was dried under nitrogen flow and redissolved in acetonitrile. The ionizing method was electrospray using negative ions; multiple reaction monitoring was used for mass analysis.

To assure quality control (QC), the precision of laboratory measurement was assessed before and after each assay using a pooled blood sample from healthy volunteers. Based on 20 replicated measurements of the QC sample at a mean concentration of 122.1 ng/mL for genistein, 88.0 ng/mL for daidzein, 10.6 ng/mL for glycitein, and 39.6 ng/mL for equol, the coefficients of variation were 3.0% or less for intraday variation and 3.9% or less for interday variation. Cases and matched controls were assayed in the same batch. Detection limits were less than 1.0 ng/mL for all isoflavones. All samples were analyzed at a single laboratory (SRL, Tokyo, Japan) while blinded to case–control status.

Statistical analysis

Baseline characteristics between cases and controls were evaluated by the Mantel–Haenszel procedure with

matched-set strata (32). For genistein and daidzein, study participants were classified into quintiles according to plasma concentration. For glycitein and equol, the lowest category comprised study participants with amounts below the detection limit (<1.0 ng/mL), and those with detectable concentrations were divided into quartiles. Total isoflavones was defined as the sum of genistein, daidzein, glycitein, and equol concentrations and was classified by quintile of plasma concentration. Glycitein and equol concentrations below the detection limit were regarded as zero in the calculation of total isoflavones. Cutoff points for plasma isoflavone concentration were based on the control distribution.

We used a conditional logistic regression model to estimate odds ratios (OR) and 95% CIs of lung cancer risk by category of plasma isoflavones and to adjust for potentially confounding variables. Dummy variables were created for the categories of plasma isoflavone concentration, and the lowest category was used as the reference category. We calculated P values for the analysis of linear trends by assigning ordinal values for categories of plasma isoflavone concentration and entering the number as a continuous term in the regression model. All reported P values are 2-tailed. All statistical analyses were performed using SAS statistical software, version 9.1 (SAS Institute Inc; ref. 33).

Multivariate-adjusted ORs were adjusted for family history of lung cancer (yes or no), pack-years of smoking among current smokers (1–19 or ≥ 20 pack-years, as defined by multiplying the years of smoking by the average number of cigarettes per day and dividing by 20), passive smoke exposure at work (≤ 1 –3 days/month, 1–4 days/week, or almost daily), past or current use of exogenous female hormones (yes or no), and fruit and vegetable intake (continuous variable). All analyses were repeated after excluding participants who received a diagnosis of lung cancer within 3 years of blood collection ($n = 20$).

Results

The characteristics of cases and controls are shown in Table 1. The prevalence of never smokers among both cases and control was 92.9% ($n = 117$ and $n = 234$, respectively). We found no significant differences in the characteristics of cases and controls. Table 2 shows plasma isoflavone concentrations in cases and controls. The median plasma concentrations of genistein, daidzein, glycitein, equol, and total isoflavones in cases were all slightly lower than those in controls; however, the differences were not statistically significant.

Table 3 shows the associations between plasma isoflavone concentrations and risk of lung cancer. After adjustment for potential confounders, there was a U-shaped association between plasma isoflavone concentrations and lung cancer risk. However, after exclusion of the 20 lung cancer cases diagnosed in the first

Table 1. Baseline characteristics of cases and controls

Characteristic	Cases	Controls	<i>P</i> ^a
	(<i>n</i> = 126)	(<i>n</i> = 252)	
Age, mean (SD), y	57.3 (7.4)	57.0 (7.3)	-
Family history of lung cancer, <i>n</i> (%)	4 (3.2)	3 (1.2)	0.18
Never smokers, <i>n</i> (%)	117 (92.9)	234 (92.9)	-
Current smokers, <i>n</i> (%)	8 (0.1)	16 (0.1)	-
1–19 pack years, <i>n</i> (%) ^b	5 (31.3)	5 (62.5)	0.15
Passive smoke exposure, almost daily, <i>n</i> (%)	24 (19.5)	46 (18.5)	0.41
Nondrinkers, <i>n</i> (%)	98 (78.4)	209 (82.9)	0.33
Postmenopausal status, <i>n</i> (%)	101 (82.8)	200 (82.0)	0.39
Age at menarche, mean (SD), y ^c	15.8 (2.0)	15.9 (2.1)	0.34
Age at menopause, mean (SD), y ^c	48.7 (4.5)	49.3 (4.0)	0.56
Past or current use of exogenous female hormones, <i>n</i> (%)	19 (17.1)	26 (11.8)	0.21
Dietary intake ^d			
Total energy, mean (SE), kcal/d	1278 (37.5)	1249 (26.5)	0.49
Vegetables, mean (SE), g/d	118 (6.6)	119 (4.6)	0.98
Fruit, mean (SE), g/d	102 (8.7)	106 (6.2)	0.70
Fish, mean (SE), g/d	44 (2.6)	49 (18)	0.14

^a*P* value on Mantel-Haenszel test with matched-set strata.^bAmong current smoking women.^cAmong postmenopausal women.^dAdjusted for cohort.

3 years after blood collection, we found an inverse association between plasma genistein concentration and lung cancer risk. After adjustment for family history of lung cancer, pack-years of smoking among current smokers, passive smoke exposure at work, past or current use of exogenous female hormones, and fruit and vegetable intake, the multivariate-adjusted ORs (95% CIs) of lung cancer across increasing quintiles of plasma genistein, with the lowest quintile as reference, were 1.00, 0.27 (0.10, 0.75), 0.21 (0.08, 0.59), 0.24 (0.08, 0.71), and 0.31 (0.12, 0.86) (*P* for trend = 0.085). For

daidzein, glycitein, and total isoflavones, the ORs of lung cancer were also below unity, but were not statistically significant. We found no association between plasma equol concentration and lung cancer risk, even after lung cancer cases in the first 3 years after blood collection were excluded.

For the purpose of sensitivity analysis, we included additional variables in the model, namely, menopausal status (premenopausal or postmenopausal), ages at menarche (<16 or ≥16 years) and menopause (≤50 or >50 years) among postmenopausal women, and

Table 2. Plasma isoflavone concentrations in cases and controls

Isoflavone	Cases (<i>n</i> = 126)		Controls (<i>n</i> = 252)		<i>P</i> ^a
	Median, ng/mL	IQR	Median, ng/mL	IQR	
Genistein	72.0	(25.4–163.1)	72.4	(29.8–127.0)	0.84
Daidzein	29.3	(9.9–66.3)	31.8	(11.6–61.4)	0.82
Glycitein ^b	1.8	(0–4.1)	2.1	(0–4.1)	0.34
Equol ^b	2.8	(0–20.2)	3.5	(0–15.4)	0.78
Total isoflavones ^c	124.7	(42.2–267.1)	126.4	(51.8–214.6)	0.82

Abbreviation: IQR, interquartile range.

^a*P* value on Mantel-Haenszel test with matched-set strata.^bValues below the detection limit (<1 ng/ml) were regarded as zero.^cTotal isoflavones is the sum of genistein, daidzein, glycitein, and equol concentrations.

Table 3. ORs and 95% CIs of lung cancer, by plasma isoflavone concentration^a

Plasma concentration	Quintile of Plasma Isoflavone Concentration ^b					P for trend
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	
Genistein, ng/mL	<24.8	24.8–52.3	52.4–88.7	88.8–151.2	>151.2	
No. of cases	34	21	19	17	35	
No. of controls	50	51	50	51	50	
OR1 (95% CI) ^c	1.00 (Reference)	0.51 (0.25, 1.07)	0.47 (0.23, 0.99)	0.43 (0.20, 0.93)	0.88 (0.45, 1.74)	0.915
OR2 (95% CI) ^d	1.00 (Reference)	0.40 (0.17, 0.94)	0.36 (0.15, 0.86)	0.36 (0.14, 0.93)	0.68 (0.30, 1.53)	0.700
OR3 (95% CI) ^e	1.00 (Reference)	0.27 (0.10, 0.75)	0.21 (0.08, 0.59)	0.24 (0.08, 0.71)	0.31 (0.12, 0.86)	0.085
Daidzein, ng/mL	<8.3	8.3–21.7	21.8–40.7	40.8–72.2	>72.2	
No. of cases	31	24	22	19	30	
No. of controls	50	51	50	51	50	
OR1 (95% CI) ^c	1.00 (Reference)	0.71 (0.35, 1.40)	0.68 (0.34, 1.34)	0.57 (0.27, 1.18)	0.94 (0.47, 1.86)	0.709
OR2 (95% CI) ^d	1.00 (Reference)	0.81 (0.37, 1.76)	0.84 (0.39, 1.82)	0.56 (0.23, 1.36)	1.03 (0.46, 2.29)	0.874
OR3 (95% CI) ^e	1.00 (Reference)	0.79 (0.34, 1.86)	0.56 (0.23, 1.36)	0.35 (0.13, 0.97)	0.73 (0.29, 1.82)	0.258
Glycitein, ng/mL	<1.0	1.0–1.9	2.0–3.0	3.1–5.4	>5.4	
No. of cases	48	15	22	22	19	
No. of controls	82	42	41	45	42	
OR1 (95% CI) ^c	1.00 (Reference)	0.59 (0.29, 1.20)	0.90 (0.48, 1.67)	0.80 (0.42, 1.51)	0.74 (0.36, 1.49)	0.513
OR2 (95% CI) ^d	1.00 (Reference)	0.42 (0.19, 0.95)	0.94 (0.47, 1.88)	0.79 (0.35, 1.79)	0.72 (0.32, 1.64)	0.601
OR3 (95% CI) ^e	1.00 (Reference)	0.42 (0.18, 1.03)	0.77 (0.36, 1.63)	0.44 (0.17, 1.19)	0.52 (0.21, 1.31)	0.147
Equol, ng/mL	<1.0	1.0–4.3	4.4–12.1	12.2–26.7	>26.8	
No. of cases	53	15	14	23	21	
No. of controls	99	38	39	38	38	
OR1 (95% CI) ^c	1.00 (Reference)	0.73 (0.36, 1.48)	0.67 (0.33, 1.37)	1.13 (0.61, 2.11)	1.03 (0.53, 2.00)	0.796
OR2 (95% CI) ^d	1.00 (Reference)	0.73 (0.32, 1.66)	0.82 (0.35, 1.94)	0.94 (0.44, 2.01)	1.08 (0.51, 2.31)	0.845
OR3 (95% CI) ^e	1.00 (Reference)	0.86 (0.35, 2.12)	0.78 (0.32, 1.92)	0.97 (0.43, 2.20)	1.07 (0.47, 2.44)	0.889
Total isoflavones, ng/mL	<42.1	42.1–86.0	86.1–148.1	148.2–257.1	>257.1	
No. of cases	30	20	21	20	35	
No. of controls	50	51	50	51	38	
OR1 (95% CI) ^c	1.00 (Reference)	0.57 (0.26, 1.25)	0.63 (0.31, 1.30)	0.56 (0.25, 1.25)	1.06 (0.52, 2.17)	0.590
OR2 (95% CI) ^d	1.00 (Reference)	0.59 (0.24, 1.49)	0.61 (0.26, 1.41)	0.58 (0.22, 1.57)	0.95 (0.41, 2.20)	0.729
OR3 (95% CI) ^e	1.00 (Reference)	0.46 (0.16, 1.34)	0.43 (0.16, 1.12)	0.41 (0.13, 1.29)	0.55 (0.20, 1.49)	0.442

^aA conditional logistic regression model was used to estimate ORs and 95% CIs.

^bFor genistein and daidzein, study participants were classified into quintiles according to plasma concentration. For glycitein and equol, the lowest category (Q1) comprised study participants with concentrations below the detection limit (<1.0 ng/mL); those with detectable concentrations were divided into quartiles.

^cMatched variables were age, public health center area, geographic area (city, or town and village), date on which blood was collected, time of day of blood collection, duration of fasting at blood collection, and smoking status.

^dOR2 was adjusted for family history of lung cancer (yes or no), pack-years of smoking among current smokers (1–19 or >20 pack-years, defined by multiplying the years of smoking by the average number of cigarettes per day and dividing by 20), passive smoke exposure at work (<1–3 days/month, 1–4 days/week, or almost daily), past or current use of exogenous female hormones (yes or no), and fruit and vegetable intake (continuous variable).

^eOR3 was adjusted for the same variables as OR2, after exclusion of lung cancer cases diagnosed in the first 3 years after blood collection.

fish intake. The results were similar (data not shown). The findings were also similar when the analysis was limited to never smokers: after exclusion of lung cancer cases in the first 3 years, the multivariate-

adjusted OR (95% CI) for the highest quintile of genistein concentration versus the lowest quintile was 0.36 (0.13–0.98; *P* for trend = 0.151), when the analysis was restricted to women who provided a fasting

blood sample (i.e., 6 or more hours after a meal), and when only participants with lung adenocarcinoma or NSCLC (adenocarcinoma, squamous cell carcinoma, or large cell carcinoma) were defined as cases (data not shown).

Discussion

In this nested case-control study within a large-scale, population-based, prospective study of Japanese women, we found that plasma concentrations of genistein, but not daidzein, glycitein, equol, or total isoflavones, were associated with a significant decrease in lung cancer risk after exclusion of lung cancer cases diagnosed within 3 years of blood collection. At the time of blood collection, participants who later developed early lung cancer might have had preclinical lung cancer, which could have changed their dietary behavior. Also, if participants with preclinical lung cancer were more likely due to ill health to have health checkups than the apparently healthy population at the baseline, they would be more likely to be cases. If indeed this occurred, any association would be distorted. We consider that the results obtained after excluding these early lung cancer cases suggest a preventive effect of genistein on lung cancer incidence. To our knowledge, this is the first study to investigate the association between plasma isoflavone concentrations and lung cancer risk.

We did not find a dose-response relationship between plasma genistein concentration and lung cancer risk, as lung cancer risk remained constant across the second through the fifth quintiles of plasma genistein concentration. Although we cannot characterize the shape of the exposure-disease relation because of the limited number of cases, the results suggest that a low genistein concentration is important in lung carcinogenesis. However, further study of a larger number of lung cancer cases is needed to confirm this hypothesis.

We observed an inverse association only for genistein. If isoflavones have an effect via estrogen-dependent mechanisms, this inverse association with genistein is plausible, as it has been reported to have greater estrogenic activity (34-36) than daidzein. Reports have shown that equol has even higher estrogenic activity than genistein (34, 36); however, the median plasma concentration of genistein in controls was 2.3 to 34.5 times that of other isoflavones, including equol (Table 2), which may explain why we failed to detect an association with isoflavones other than genistein.

In addition to the estrogen receptor-mediated mechanism, we speculate that a mechanism mediated by the epidermal growth factor receptor (EGFR) may be involved. The EGFR mediates signals related to increased cell proliferation and inhibition of apoptosis (37). While mutations in the *EGFR* gene activate the EGFR pathway (38), NSCLC with mutated *EGFR*

is highly responsive to gefitinib, an EGFR protein-tyrosine kinase (PTK) inhibitor (39). Interestingly, genistein is reported to be a PTK inhibitor, based on the fact that it inhibited EGFR PTK activity *in vitro* (40). Genistein inhibited growth of NSCLC cell lines, particularly one with mutated *EGFR* (16). Furthermore, a case-control study in Japan found that soy food intake was inversely associated with *EGFR*-mutated NSCLC only (41). Although information was not available on the *EGFR* status of lung cancer in our study, the present participants had characteristics similar to those associated with the *EGFR* mutation, that is, never-smoking status, East Asian ethnicity, and female sex (42). Genistein might exert its preventive effect on lung cancer through the *EGFR*-mediated mechanism.

Only 2 prospective studies have examined the association between isoflavone intake and lung cancer risk in Asian countries, where isoflavone intake is higher than in Western countries. We previously reported an association between isoflavone intake and lung cancer risk, using data from a 5-year follow-up questionnaire in our cohort (20). In that study, we found a nonsignificant inverse association between isoflavone intake (determined by using genistein intake) and lung cancer risk in women (hazard ratio for the highest vs. lowest quartile of intake: 0.83; 95% CI: 0.54, 1.29; *P* for trend = 0.409). In the Singapore Chinese Health Study, Seow and colleagues reported an inverse association between isoflavone intake and overall risk of lung cancer in nonsmoking women: the multivariate-adjusted hazard ratio for lung cancer incidence in the highest versus the lowest quartile of isoflavone intake was 0.59 (95% CI: 0.38, 0.91) (19). These findings conform to those of the current study.

The limitations of this study warrant mention. First, we used a single measurement of plasma isoflavone concentration, which may be subject to day-to-day and diurnal variation. However, in a validation study using a subsample of the cohort, high reproducibility of genistein intake was observed: the correlation coefficients for FFQ estimates separated by 1 year and 5 years were 0.72 (43) and 0.61 (28), respectively. Furthermore, our validation study yielded satisfactorily high correlation coefficients for genistein estimates from dietary records (DR) measured repeatedly for a year, a fasting serum sample, and a single FFQ (DR vs. serum: 0.33; DR vs. FFQ: 0.59; ref. 43). As isoflavone intake was likely to have been stable for a long period in this population, we consider it unlikely that day-to-day variation in plasma isoflavone concentrations substantially distorted the association between plasma isoflavone concentration and lung cancer risk. Because of the half-life of genistein and daidzein in blood (7.7 to 9.5 hours; ref. 44), plasma concentrations of isoflavones vary with regard to fasting time. To minimize attenuation in risk estimation due to diurnal variation, fasting time was matched in cases and controls. A second

limitation of the study was that, due to the relatively small number of lung cancer cases among ever smokers ($n = 9$), it was not possible to conduct stratified analyses by smoking status. Third, the current nested case-control study was conducted among health check-up participants, a subsample of the entire cohort that had different background characteristics from nonparticipants in health check-ups (45). Female participants in health check-ups had a favorable lifestyle profile. As compared with nonparticipants, they smoked and drank less, but tended to eat fruit and green vegetables more often and to participate more in sports and physical exercise in their leisure time. Thus, the associations between plasma isoflavone concentrations and lung cancer risk could differ from those of the entire cohort. However, because there was a similar inverse association between lung cancer and isoflavone in both the current participants and the entire cohort (20), the findings of the current study are not likely to be substantially biased as compared with those of the entire cohort.

Our study has several strengths. First, we directly measured plasma isoflavone concentrations, which reflect absorption and metabolism. Second, collecting blood samples before a diagnosis of lung cancer enabled us to infer a protective effect of genistein on lung cancer risk. Third, the quality of information measured at baseline was comparable in cases and controls, because both were selected from the same cohort.

In conclusion, plasma genistein concentration was associated with a decreased risk of lung cancer in Japanese women.

Appendix

Members of the JPHC Study Group (principal investigator: S. Tsugane): S. Tsugane, M. Inoue, T. Sobue, and T. Hanaoka, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo; J. Ogata, S. Baba, T. Mannami, A. Okayama, and Y. Kokubo, National Cardiovascular Center, Suita; K. Miyakawa, F. Saito, A. Koizumi, Y. Sano, I. Hashimoto, T. Ikuta, and Y. Tanaba, Iwate Prefectural Ninohe Public Health Center, Ninohe; Y. Miyajima, N. Suzuki, S. Nagasawa, Y. Furusugi, and N. Nagai, Akita Prefectural Yokote Public Health Center, Yokote; H. Sanada, Y. Hatayama, F. Kobayashi, H. Uchino, Y. Shirai, T. Kondo, R. Sasaki, Y. Watanabe, Y. Miyagawa, Y. Kobayashi, and M. Machida, Nagano Prefectural Saku Public Health Center, Saku; Y. Kishimoto, E. Takara, T. Fukuyama, M. Kinjo, M. Irei, and H. Sakiyama, Okinawa Prefectural Chubu Public Health Center, Okinawa; K. Imoto, H. Yazawa, T. Seo, A. Seiko, F. Ito, F. Shoji, and R. Saito, Katsushika Public Health Center, Tokyo; A. Murata, K. Minato, K. Motegi, and T. Fujieda, Ibaraki Prefectural Mito Public Health Center, Mito; T. Abe, M. Katagiri, M. Suzuki, and K. Matsui, Niigata Prefectural Kashiwazaki and Nagaoka

Public Health Center, Kashiwazaki and Nagaoka; M. Doi, A. Terao, Y. Ishikawa, and T. Tagami, Kochi Prefectural Chuo-higashi Public Health Center, Tosayamada; H. Doi, M. Urata, N. Okamoto, F. Ide, and H. Sueta, Nagasaki Prefectural Kamigoto Public Health Center, Arikawa; H. Sakiyama, N. Onga, H. Takaesu, and M. Uehara, Okinawa Prefectural Miyako Public Health Center, Hirara; F. Horii, I. Asano, H. Yamaguchi, K. Aoki, S. Maruyama, M. Ichii, and M. Takano, Osaka Prefectural Suita Public Health Center, Suita; S. Matsushima and S. Natsukawa, Saku General Hospital, Usuda; M. Akabane, Tokyo University of Agriculture, Tokyo; M. Konishi, K. Okada, and I. Saito, Ehime University, Toon; H. Iso, Osaka University, Suita; Y. Honda, K. Yamagishi, S. Sakurai, and N. Tsuchiya, Tsukuba University, Tsukuba; H. Sugimura, Hamamatsu University, Hamamatsu; Y. Tsubono, Tohoku University, Sendai; M. Kabuto, National Institute for Environmental Studies, Tsukuba; S. Tominaga, Aichi Cancer Center Research Institute, Nagoya; M. Iida, W. Ajiki, and A. Ioka, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka; S. Sato, Osaka Medical Center for Health Science and Promotion, Osaka; N. Yasuda, Kochi University, Nankoku; K. Nakamura, Niigata University, Niigata; S. Kono, Kyushu University, Fukuoka; K. Suzuki, Research Institute for Brain and Blood Vessels Akita, Akita; Y. Takashima and M. Yoshida, Kyorin University, Mitaka; E. Maruyama, Kobe University, Kobe; M. Yamaguchi, Y. Matsumura, S. Sasaki, and S. Watanabe, National Institute of Health and Nutrition, Tokyo; T. Kadowaki, Tokyo University, Tokyo; M. Noda and T. Mizoue, International Medical Center of Japan, Tokyo; Y. Kawaguchi, Tokyo Medical and Dental University, Tokyo; and H. Shimizu, Sakihae Institute, Gifu.

Disclosure of Potential Conflicts of Interests

No potential conflicts of interest were disclosed.

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Tutorial videos of bioinformatics resources: online distribution trial in Japan named TogoTV

Shin Kawano, Hiromasa Ono, Toshihisa Takagi and Hidemasa Bono

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Abstract

In recent years, biological web resources such as databases and tools have become more complex because of the enormous amounts of data generated in the field of life sciences. Traditional methods of distributing tutorials include publishing textbooks and posting web documents, but these static contents cannot adequately describe recent dynamic web services. Due to improvements in computer technology, it is now possible to create dynamic content such as video with minimal effort and low cost on most modern computers. The ease of creating and distributing video tutorials instead of static content improves accessibility for researchers, annotators and curators. This article focuses on online video repositories for educational and tutorial videos provided by resource developers and users. It also describes a project in Japan named TogoTV (<http://togotv.dbcls.jp/en/>) and discusses the production and distribution of high-quality tutorial videos, which would be useful to viewer, with examples. This article intends to stimulate and encourage researchers who develop and use databases and tools to distribute how-to videos as a tool to enhance product usability.

Keywords: screencast; vodcast; tutorial; YouTube; QuickTime; Flash

INTRODUCTION

Recent advances in life sciences technology have dramatically changed the research style from hypothesis-driven research (bottom-up style) to data-driven research (top-down style). Current 'omics' projects have produced vast amounts of data that have been stored in various online databases. Simultaneously, many types of web tools have been developed to analyze the stored data. Some of them are annually featured in the *Nucleic Acid Research's* database issue and web server issue [1, 2]. Although the increase in available resources (databases and tools) has promoted life sciences research, this situation causes the following difficulties for researchers, especially

'wet' biologists: (i) What kinds of resources exist? (ii) Where are they? (iii) How can the resources be used and combined? and (iv) How does one interpret a result? To solve these issues, development of educational content as well as a system for navigation of web resources is required [3].

Traditional methods for distributing educational content include publishing textbooks and web documents. Although the contents of a textbook are sustainable, they quickly become obsolete because of frequent updates of web interfaces and improvement in web service functions. Web documents can more easily keep up with database and tool updates. However, it has become difficult to describe current

Corresponding author. Hidemasa Bono, Database Center for life science, Research Organization of Information and Systems, 2-11-16 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan. Tel: +81-3-5841-7957; Fax: +81-3-5841-8091; E-mail: togo-sohshi@dbcls.rois.ac.jp

Shin Kawano is a Project Researcher at Database Center for Life Science (DBCLS). He produces TogoTV contents, maintains its web site and organizes workshops. His research focuses on bioinformatics approach to proteomics and glycomics.

Hiromasa Ono is a Project Senior Technical Support Specialist at DBCLS. He produces TogoTV contents and dictates research assistants. His research focuses on cellular dedifferentiation and acquisition of multipotency.

Toshihisa Takagi is a Professor at the University of Tokyo and the National Institute of Genetics. He is the head of the Integrated Database Project. His research interests include bioinformatics, ontology, text mining and database architecture.

Hidemasa Bono is a Project Associate Professor at DBCLS. He designed TogoTV project. He also develops gene expression database and analyzing tools. He is a specialist of transcriptome analysis.

web services in such static documents because of the evolution of web technology. Web 2.0, Flash and AJAX (Asymmetric JavaScript + XML) have led to the development of interactive and dynamic web services. This type of content would be better expressed in an animated environment rather than in a document. As a similar example, educational videos have been distributed using videotapes, CDs/DVDs and the Internet for >30 years in some clinical fields because it was excessively difficult to describe an actual procedure in writing [4–7]. However, their creation and distribution costs were high.

Rapid improvements in recent years in computer hardware, software and the Internet have reduced the publishing cost of multimedia content. A personal computer with a high-end CPU, extensive memory and large-capacity storage space enables users to produce and encode videos with relative ease. The latest releases of major OS packages include software for recording, editing and encoding videos, such as Windows Live Movie Maker (Microsoft Corporation, Redmond, WA, USA) and QuickTime (Apple Inc., Cupertino, CA, USA). The reasonable price of such software reduces the installation cost, and its user friendliness reduces the time required. In addition to the reduction in production cost, broadband networks have also reduced the distribution cost of multimedia content and have allowed experts to readily distribute video content in their field to anyone with an Internet connection. Many recent web browsers are by default equipped with video players, such as the Adobe Flash player (Adobe Systems Incorporated, San Jose, CA, USA) and QuickTime player (Apple Inc.); thus, one can easily view a video on web browser by simply clicking the play button.

In this article, we describe online video repositories for educational purposes, worldwide movements of distributing video tutorials created by major database and tool developers, and our recent activity in Japan. In addition, we propose distributing how-to videos to the developers and users of databases and tools to promote their usability and contribute to the scientific community.

ONLINE VIDEO REPOSITORIES

Several web services are already available for video distribution. YouTube is the most popular online video sharing service, and it contains many tutorial

videos and lectures in many fields [8]. Similarly, there are repository services such as Dailymotion and Vimeo (for more examples, see the Wikipedia article entitled ‘List of video hosting services’) [9–11]. Most services are free to use, and any registered user can upload video. Live streaming services such as Ustream, Justin.tv and Stickam also exist [12–14]. As the term ‘live streaming’ suggests, these services provide live streaming services for lectures, workshops, seminars and meetings that are recorded and may be played back at a later time.

In the scientific field, the *Journal of Visualized Experiments* has been published since 2006 [15]. It is a peer-reviewed, PubMed-indexed journal devoted to the publication of biological research in a video format. SciVee offers a comprehensive set of rich media solutions to enhance the discovery and collaboration of knowledge [16]. It provides Video and Podcasts (standard videos and podcasts), PubCast (synchronized video abstracts of peer-reviewed articles), PaperCast (synchronized video abstracts of non-peer-reviewed articles), SlideCast (synchronized videos of slide presentations) and PosterCast (synchronized videos of posters or other conference presentations) in collaboration with scientists and researchers, as well as journals and publishers, societies, conference organizers, universities and research institutions. Dnatube is a community-based repository of scientific videos including educational materials, seminars and lectures [17]. This site has over 5000 videos and 30 000 community members. Individual videos can be found using keyword search, category tags and topics.

Some universities and organizations also administer a video repository server, especially for providing lecture videos that are part of OpenCourseWare (OCW). The Massachusetts Institute of Technology (MIT) hosts MIT OCW and MIT World, and the University of Tokyo provides UT OCW [18–20]. Academic Earth provides online courses of the world’s top scholars from Harvard University and Stanford University among other top academic institutions [21]. YouTube also has a special channel for education from colleges and universities named YouTube EDU, and another channel, Technology, Entertainment, Design (TED), delivers interesting lectures by respected individuals [22, 23]. A complete list of OCW websites is found at the OCW Consortium Website, and other useful services are listed in the Wikipedia article entitled ‘List of educational video websites’ [24, 25].

In addition to repository-type services, delivery-type services named vodcasts (video podcasts) are available via Really Simple Syndication (RSS) technology. If a user subscribes to a vodcast program in a vodcast player such as iTunes, the contents of the program are automatically updated when new content arrives. Since the vodcast programs can be transferred to portable devices such as the iPod, iPhone or iPad, the user can watch them anytime, anywhere. Although vodcast programs are mainly focused on news, entertainment and fashion, educational programs are also provided. Indeed, some institutes have already used the podcast/vodcast for education [26–28]. Apple collects and webcasts educational contents via the iTunes store called iTunes U [29].

VIDEO TUTORIALS PROVIDED BY RESOURCE DEVELOPERS AND USERS

As noted earlier, the publishing of tutorial videos by some providers has increased as the creation and distribution costs of videos have decreased. For example, National Center for Biotechnology Information provides tutorial videos of some services both on the YouTube channel and on their server such as dbGaP, the database of Genotypes and Phenotypes, that archives and distributes the results of studies that have investigated the interaction of genotype and phenotype and PubMed that is a database of citations and abstracts for biomedical literature from MEDLINE and additional life sciences journals [30–35]. Some projects in the European Bioinformatics Institute also distributed how-to videos for tools such as Ensembl that is genome databases for vertebrates and other eukaryotic species, QuickGO that is a fast web-based browser for Gene Ontology (GO) terms and annotations, and GOA, Gene Ontology Annotation, that provides high-quality GO annotations to proteins in the UniProt Knowledgebase and International Protein Index [36–41].

Not only service providers in national institutes but also individual service providers including relatively small communities distributed tutorial videos. Galaxy, a collaboration system for genomic research, is a highly functional and complex system, but the procedure is easily understandable because the developers provide tutorial videos on their website [42, 43]. Taverna, which is an open source and domain-independent workflow management system

(a suite of tools used to design and execute scientific workflows and aid *in silico* experimentation), is also described in the tutorials in a video format [44, 45]. ATTED-II, which provides co-regulated gene relationships to estimate gene function, has YouTube channel for tutorials [46, 47]. There are many video tutorials provided by the database and tool developers.

In addition, educators and users of web resources who do not develop any databases or tools also contribute to the scientific community by providing tutorial videos. BITS, Bioinformatics Tutorials Series, is a collaboration work of the MIT Engineering and Science Libraries and Harvard's Countway Library [48, 49]. BIREC, Bioinformatics Information Resource and eLearning Center, also provides tutorial videos [50]. OpenHelix provides over 100 well-organized tutorial suites including videos on web-based bioinformatics and genomic resources [3, 51]. It also has many tutorial videos in 'Tip of this week' tagging articles in the blog section [52]. In addition to videos provided by organizations, a YouTube search by database or tool name will provide many tutorial videos produced by volunteers.

TogoTV: ONLINE TUTORIAL VIDEO DISTRIBUTION TRIAL IN JAPAN

To bridge the gap between service providers and users, we created and distributed tutorial videos of databases and web tools. We describe in this article, a methodology for making and distributing videos and elaborate on this methodology with examples. TogoTV ('Togo' means 'integration' in Japanese; pronunciation symbol is [tougoʊ]) that is one of the services in the Integrated Database Project in Japan (Figure 1) is a portal site of tutorial and lecture videos about bioinformatics resources [53–55]. Although the original TogoTV site is mostly written in Japanese [53], there is the English interface for international users [54]. The site contains our original videos and third-party videos from publicly available website such as YouTube. All contents provided by us are distributed under the Creative Commons Attribution 2.1 Japan license and also provided as vodcasts that can be viewed using a portable device and on YouTube. Although most of the contents are described in Japanese, there are 19 original programs in English, most of which explain a service developed in the Integrated Database Project such