

## Influence of Drinking Green Tea on Breast Cancer Malignancy among Japanese Patients

Kei Nakachi,<sup>1,3</sup> Kimito Suemasu,<sup>2</sup> Kenji Suga,<sup>1</sup> Takeshi Takeo,<sup>2</sup> Kazue Imai<sup>1</sup> and Yasuhiro Higashi<sup>2</sup>

<sup>1</sup>Department of Epidemiology, Saitama Cancer Center Research Institute and <sup>2</sup>Department of Breast Surgery, Saitama Cancer Center Hospital, 818 Komuro, Ina, Saitama 362

**Inhibitory effects of green tea on carcinogenesis have been investigated in numerous laboratory studies using (–)-epigallocatechin gallate (EGCG) or crude green tea extract, and there is also some epidemiologic evidence. Further, EGCG has been reported to inhibit the growth of cancer cells, lung metastasis in an animal model, and urokinase activity. In this study, we first examined the association between consumption of green tea prior to clinical cancer onset and various clinical parameters assessed at surgery among 472 patients with stage I, II, and III breast cancer. We found that increased consumption of green tea was closely associated with decreased numbers of axillary lymph node metastases among premenopausal patients with stage I and II breast cancer and with increased expression of progesterone receptor (PgR) and estrogen receptor (ER) among postmenopausal ones. Since these are potential prognostic factors, we then investigated the prognosis of breast cancer with special reference to consumption of green tea, in a follow-up study of these patients. We found that increased consumption of green tea was correlated with decreased recurrence of stage I and II breast cancer ( $P < 0.05$  for crude disease-free survival); the recurrence rate was 16.7 or 24.3% among those consuming  $\geq 5$  cups or  $\leq 4$  cups per day, respectively, in a seven-year follow-up of stage I and II breast cancer, and the relative risk of recurrence was 0.564 (95% confidence interval, 0.350–0.911) after adjustment for other lifestyle factors. However, no improvement in prognosis was observed in stage III breast cancer. Our results indicate that increased consumption of green tea prior to clinical cancer onset is significantly associated with improved prognosis of stage I and II breast cancer, and this association may be related to a modifying effect of green tea on the clinical characteristics of the cancer.**

Key words: Breast cancer — Green tea — Prognosis — Follow-up study

Breast carcinoma is one of the most common cancers among women in developed countries. In western countries, one in ten women will develop breast cancer. Although breast cancer is rapidly increasing in Japan, the incidence is low compared with that in western countries: one in forty Japanese women will develop the cancer.

Our prospective cohort study in a Japanese population indicated that drinking green tea, an integral part of the Japanese lifestyle, significantly prevents incidence of all-site cancer (as well as cardiovascular disease),<sup>1,2)</sup> and a recent site-specific analysis of the cohort study data suggests that consumption of large amounts of green tea prevents breast cancer (data not shown). The putative cancer-preventive effects of green tea on carcinogenesis have been investigated by numerous laboratory studies using EGCG, the main constituent of tea polyphenols, or using crude green tea extract.<sup>3–13)</sup> Concerning breast carcinogenesis, EGCG suppressed aberrant hyperproliferation in *c-myc*-transfected and murine mammary tumor virus-initi-

ated mammary epithelial cells.<sup>14)</sup> Very recently, in an experiment using [<sup>3</sup>H]EGCG, significant radioactivity was found in the target organs of EGCG and green tea extract (the digestive tract, liver, lung, pancreas, and skin), as well as in other organs including mammary gland and ovary, after administration into the stomach of mouse.<sup>15)</sup> These results not only explain the inhibitory effects of green tea on carcinogenesis in various organs observed so far, but also strongly suggest that it may have inhibitory effects in other organs not yet studied. Besides the inhibition of carcinogenesis, EGCG also inhibited the growth of several cancer cell lines, including a breast cancer cell line, MCF-7.<sup>6)</sup> Further, EGCG in drinking water inhibited lung metastasis of B16 melanoma cells in experimental and spontaneous mouse systems.<sup>16)</sup> Very recently, inhibition of urokinase activity by EGCG was reported, suggesting that green tea may influence the malignancy of tumor cells, including invasion and metastasis.<sup>17)</sup> These results indicate that green tea may have multiple functions against carcinogenesis and also against cancer cells, raising the possibility that malignant phenotypes of cancer might be influenced by consumption levels of green tea among patients.

<sup>3</sup> To whom all correspondence should be addressed.

Abbreviations used: EGCG, (–)-epigallocatechin gallate; PgR, progesterone receptor; ER, estrogen receptor.

In this study, we first examined the association between consumption of green tea and various clinical parameters assessed at surgery among Japanese breast cancer patients, a considerable percentage of whom daily consumed large amounts of green tea. Second, the prognosis of these breast cancer patients was studied with special reference to consumption levels of green tea, in a follow-up study.

#### SUBJECTS AND METHODS

Study subjects were 472 patients with histologically confirmed invasive breast carcinomas (117, 273, and 82 patients with stage I, stage II, and stage III breast cancer, respectively), who underwent partial or total mastectomy

along with interviewing for epidemiological information, at Saitama Cancer Center Hospital during 1984–1993. This epidemiological survey was carried out on all possible inpatients with cancer of all sites during this period, at the time when they first entered the hospital, and some of these epidemiological data were used for other lines of molecular epidemiological study.<sup>18–20</sup> A standardized questionnaire was used in this survey, covering height, weight, occupation, intake frequency of main beverages (green tea, coffee, black tea) and food groups (soybean paste soup and other soybean products, nuts, fruits, green and yellow vegetables, seaweed, fish, meat, eggs, and milk); cigarette consumption, passive smoking, and alcohol consumption; experience of pregnancy, child birth,

Table I. Baseline Characteristics of Breast Cancer Patients by Clinical Staging

	Stage I	Stage II	Stage III	Total
No. of patients	117	273	82	472
Mean age (years, $\pm$ SD)	48.9 $\pm$ 10.8	49.9 $\pm$ 11.3	50.5 $\pm$ 11.5	49.7 $\pm$ 11.2
Menopausal status				
Premenopausal	77 (65.8%)	159 (58.2%)	51 (62.2%)	287 (60.8%)
Postmenopausal	34 (29.1)	99 (36.3)	30 (36.6)	163 (34.5)
Perimenopausal	6 ( 5.1)	15 ( 5.5)	1 ( 1.2)	22 ( 4.7)
Histological typing				
Papillotubular	26 (22.2%)	55 (20.1%)	11 (13.4%)	92 (19.5%)
Solid-tubular	40 (34.2)	95 (34.8)	41 (50.0)	176 (37.3)
Scirrhus	44 (37.6)	105 (38.5)	25 (30.5)	174 (36.9)
Other types	7 ( 6.0)	18 ( 6.6)	5 ( 6.1)	30 ( 6.4)
Mean no. of metastasized axillary lymph nodes $\pm$ SD (No. of node negative)	0.51 $\pm$ 1.7 (89)	2.1 $\pm$ 3.9 (138)	7.4 $\pm$ 6.1 (7)	2.6 $\pm$ 4.6 (234)
Blood vessel invasion				
–	52 (44.4%)	97 (35.5%)	16 (19.5%)	165 (35.0%)
+	46 (39.3)	98 (35.9)	28 (34.1)	172 (36.4)
++	18 (15.4)	68 (24.9)	33 (40.2)	119 (25.2)
+++	1 ( 0.9)	10 ( 3.7)	5 ( 6.1)	16 ( 3.4)
Lymphatic vessel invasion				
–	41 (35.0%)	56 (20.5%)	5 ( 6.1%)	102 (21.6%)
+	38 (32.5)	97 (35.5)	9 (11.0)	144 (30.5)
++	31 (26.5)	88 (32.2)	34 (41.5)	153 (32.4)
+++	7 ( 6.0)	32 (11.7)	34 (41.5)	73 (15.5)
ER <5 fmol/mg protein	22 (18.8%)	65 (23.8%)	36 (43.9%)	123 (26.1)
$\geq$ 5	95 (81.2)	208 (76.2)	46 (56.1)	349 (73.9)
PgR <10 fmol/mg protein	44 (37.6%)	98 (35.9%)	50 (61.0%)	192 (40.7%)
$\geq$ 10	73 (62.4)	175 (64.1)	32 (39.0)	280 (59.3)
Consumption of green tea				
$\leq$ 4 cups/day	51 (43.6%)	130 (47.6%)	41 (50.0%)	222 (47.0%)
$\geq$ 5	66 (56.4)	143 (52.4)	41 (50.0)	250 (53.0)
Follow-up status				
Mean follow-up years $\pm$ SD	7.5 $\pm$ 2.3	6.7 $\pm$ 2.9	4.4 $\pm$ 3.5	6.5 $\pm$ 3.0
No. of patients with recurrence	11	68	44	123
Mean years at recurrence $\pm$ SD	2.8 $\pm$ 1.7	3.2 $\pm$ 2.3	1.9 $\pm$ 1.8	2.7 $\pm$ 2.2
Recurrence rate (%)	9.4	24.9	53.7	26.1

and abortion. The information on consumption of green tea was collected from these interview data in terms of six levels of daily consumption, i.e., ≤one cup, 2–4, 5–7, 8–10, 11–13, and ≥14 cups. The content of EGCG was estimated to be 30–40 mg per cup, according to our parallel surveys.<sup>1)</sup>

Clinical parameters considered in the analysis were age at surgery, clinical staging, tumor volume, histological typing, numbers of metastasized axillary lymph nodes, blood vessel invasion, lymphatic vessel invasion, expression levels of ER and PgR measured by enzyme immunoassay, degree of histological spread, and use of chemotherapy, radiotherapy, and tamoxifen.

Subsequently, we ran a follow-up survey in this cohort of breast cancer patients: the mean follow-up period (SD) was 6.5 years (3.0). During the follow-up period, we identified a total of 123 patients with recurrence of breast cancer (overall recurrence rate, 26.1%) and six patients, including one of those with recurrence, who contracted metachronous contralateral breast cancer. The follow-up status by clinical staging is summarized in Table I. The effects of drinking green tea on disease-free survival were evaluated using the Cox proportional hazards model (SPSS). A stepwise method was also used for backward selection of potential predictor variables within the framework of the Cox proportional hazards model.

## RESULTS

**Association between green tea consumption and clinical parameters of breast cancer** Baseline characteristics of breast cancer patients are shown in terms of selected

clinical and epidemiological variables (Table I). Age, menopausal status, and consumption of green tea are very little affected by clinical staging, while histological typing, number of metastasized axillary lymph nodes, blood and lymphatic vessel invasion, and expression of ER and PgR show significant associations with clinical staging.

Habitual consumption of green tea from an early age may influence malignant phenotypes of breast cancer which are reflected by clinical parameters assessed at surgery. We investigated this possibility by examining the association of green tea consumption with available clinical parameters: age, clinical staging, tumor volume, histological typing, number of metastasized axillary lymph nodes, degree of blood vessel invasion, degree of lymphatic vessel invasion, degree of histological spread, and expression levels of ER and PgR. In stage I and II breast cancer, increased consumption of green tea was closely associated with decreased numbers of metastasized axillary lymph nodes among premenopausal patients and increased expression of PgR and ER among postmenopausal ones (Table II). Other epidemiologic factors did not show significant association with these clinical parameters, except that age showed a significant correlation with ER, PgR, and numbers of metastasized axillary lymph nodes; in addition, body mass index had a significant correlation with ER. The association between consumption of green tea and those clinical parameters remained substantially unchanged even when consumption levels of green tea were differently categorized, or when the influence of age and body mass index was taken into account by covariance analysis. Mean consumption of green tea among those in the category of ≤4, 5–7 or ≥8 cups per day in

Table II. Association between Daily Consumption of Green Tea and Selected Clinical Parameters in Stage I and II Breast Cancer<sup>a)</sup>

	Mean no. of metastasized axillary lymph nodes (SE)			Mean expression of PgR (SE, fmol/mg protein)			Mean expression of ER (SE, fmol/mg protein)		
	Pre-menopausal	Post-menopausal	Total	Pre-menopausal	Post-menopausal	Total	Pre-menopausal	Post-menopausal	Total
≤4 cups/day	2.24 (0.38)	1.44 (0.47)	1.97 (0.28)	159.6 (23.3)	32.2 (8.4)	116.9 (16.2)	48.3 (6.9)	112.8 (22.9)	72.8 (9.0)
5 to 7 cups	1.17 (0.38)	1.20 (0.17)	1.15 (0.26)	171.9 (29.5)	91.3 (34.6)	153.0 (13.4)	51.9 (6.3)	112.9 (21.1)	93.4 (13.8)
≥8 cups/day	0.95 (0.33)	2.05 (0.34)	1.45 (0.39)	163.3 (38.8)	98.4 (22.6)	131.7 (14.9)	48.4 (13.1)	180.4 (42.2)	109.7 (21.8)
Test for trend	<i>P</i> =0.02	n.s.	n.s.	n.s.	<i>P</i> =0.06	n.s.	n.s.	n.s.	<i>P</i> =0.05

a) Numbers of study patients were 120, 75, and 41 with premenopausal breast cancer, 48, 49, and 36 with postmenopausal, and 181, 131, and 78 in total, including 21 perimenopausal patients, at consumption levels of up to 4 cups, 5 to 7 cups, and 8 cups or more per day, respectively.  
n.s., not significant.

Table II is roughly estimated to be 2, 6 or 11 cups, respectively (one, 3, 6, 9, 12, and 14 cups are assigned to the six levels,  $\leq$ one cup, 2-4, 5-7, 8-10, 11-13, and  $\geq$ 14 cups per day, used in the questionnaire, respectively). However, no significant association was found in stage III breast cancer (data not shown).

We then examined whether these clinical parameters associated with consumption of green tea influence the prognosis of breast cancer. The stepwise method for selection of potential predictors from all the clinical variables

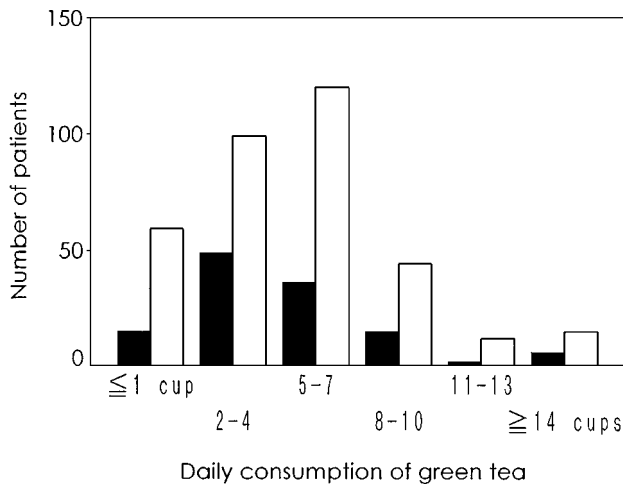


Fig. 1. Daily consumption of green tea prior to cancer onset among 349 patients without recurrence of breast cancer and 123 patients with recurrence. ■ relapsed, □ relapse-free.

revealed that the number of metastasized axillary lymph nodes and expression levels of PgR are the most consistent predictors of recurrence in stage I and II breast cancer ( $P < 0.0001$  and  $0.001$ , respectively), followed by clinical staging ( $P = 0.004$ ), histological spread ( $P = 0.01$ ), use of chemotherapy ( $P = 0.03$ ), and blood vessel invasion ( $P = 0.04$ ). Here, use of chemotherapy was associated with increased recurrence, possibly ascribable to a selection bias in applying chemotherapy to more advanced breast cancer patients. Expression of ER was not significantly influential when PgR was included in the model, in part due to a close association between them.

**Recurrence rate of breast cancer in relation to consumption of green tea** We first compared the daily consumption of green tea prior to clinical cancer onset among 349 patients without recurrence and 123 patients with recurrence (Fig. 1). The distribution of consumed amounts of green tea among those without recurrence was significantly shifted to larger amounts than that among those with recurrence ( $P < 0.05$ ), indicating the dichotomous levels of green tea consumption to be  $\leq 4$  cups and  $\geq 5$  cups per day, which discriminate the individual peaks of the two distributions in Fig. 1 and almost equally divide the patients. Mean consumption of green tea among those consuming  $\leq 4$  cups and  $\geq 5$  cups per day is estimated to be 2 or 8 cups, respectively. The crude recurrence rates were then compared in relation to the dichotomous levels of green tea consumption and clinical staging in Table III; for this purpose, stages I and II were combined because of the small number, 11, of relapsed patients in stage I. In stages I and II, the recurrence rate of 16.7% among those consuming  $\geq 5$  cups per day is lower than that of 24.3% among those consuming  $\leq 4$  cups

Table III. Recurrence Rates of Breast Cancer and Mean Disease-free Period among Patients with Recurrence by Consumption of Green Tea and Clinical Staging

	Stages I and II	Stage III	Total
Consumption of green tea			
$\leq 4$ cups/day			
Recurrence rate	24.3 (44/181)	48.8 (20/41)	28.8% (64/222)
Disease-free period (yrs, SD)	2.8 (2.2)	1.9 (2.1)	2.5 (2.2)
$\geq 5$ cups/day			
Recurrence rate	16.7 (35/209)	58.5 (24/41)	23.6% (59/250)
Disease-free period (yrs, SD)	3.6 (2.2)	1.9 (1.6)	2.9 (2.2)
All consumption levels			
Recurrence rate	20.0 (79/390)	53.7 (44/82)	26.1% (123/472)
Disease-free period (yrs, SD)	3.2 (2.2)	1.9 (1.8)	2.7 (2.2)

per day with borderline significance ( $P=0.06$ ), although the rates show no statistical significance in stage III. Crude disease-free survival in stage I and II breast cancer also showed a significant improvement with increased consumption of green tea in terms of the Cox proportional hazards model ( $P<0.05$ ).

**Influence of other lifestyle factors on recurrence of breast cancer** Since other lifestyle factors besides drinking green tea might have confounded the results in Table

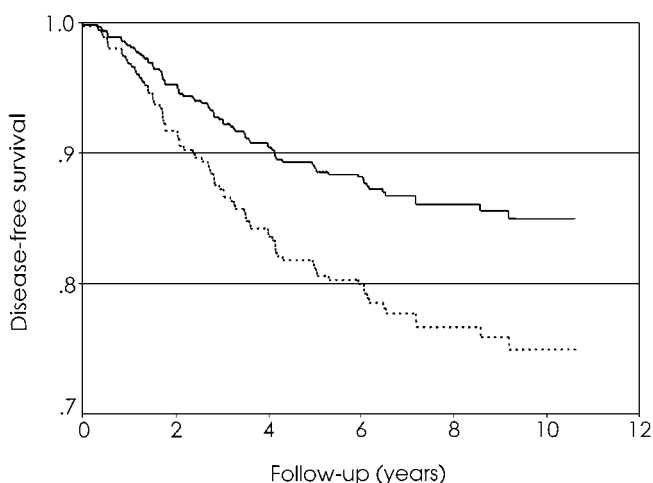


Fig. 2. Disease-free survival by daily consumption levels,  $\leq 4$  cups and  $\geq 5$  cups, of green tea, adjusted for lifestyle factors: intake of meat, fish, eggs, milk, soybean products, soybean paste soup, nuts, seaweed, fruits, green and yellow vegetables, coffee, black tea, and green tea; cigarette consumption and alcohol use; frequency of pregnancy, child birth, and abortion; body mass index and age. —  $\geq 5$  cups/day, ----  $\leq 4$  cups/day.

III, this possibility was examined in stage I and II breast cancer by the stepwise method for backward selection of potential predictor variables. As the first step for selection, we considered all possible lifestyle factors from the interview data: intake of meat, fish, eggs, milk, soybean products, soybean paste soup, nuts, seaweed, fruits, green and yellow vegetables, coffee, black tea, and green tea; cigarette consumption and alcohol use; frequency of pregnancy, child birth, and abortion; body mass index and age. After stepwise selection of variables, in which the interaction between variables was considered, we found that increased consumption of green tea remained as the only potential predictor for decreased recurrence of cancer with a statistical significance of  $P<0.05$ , followed by decreased consumption of milk ( $P=0.10$ ) and increased consumption of fruits ( $P=0.11$ ). Other variables were at very low significance levels of  $P>0.2$ , including age and body mass index. Lifestyle-adjusted disease-free survival was then estimated for the dichotomous levels of green tea consumption in Fig. 2, resulting in a significantly improved prognosis among those consuming increased amounts of green tea ( $P=0.02$ ). Relative risk of recurrence in stage I and II breast cancer was adjusted for all lifestyle factors mentioned above, and the results are summarized in Table IV, where the results for stage III breast cancer are also shown. When we included stage III breast cancer, the difference of disease-free survival for the dichotomous levels of green tea consumption was no longer statistically significant ( $P=0.15$ ).

**Disease-free survival in relation to consumption of green tea adjusted for potential clinical parameters** If increased consumption of green tea causes the improved prognosis of breast cancer by means of decreased metastasis to axillary lymph nodes and increased PgR, disease-free survival through consumption of green tea should

Table IV. Relative Risk of Recurrence of Breast Cancer for Increased Consumption of Green Tea  $\geq 5$  Cups vs.  $\leq 4$  Cups per Day

	Crude relative risk	Lifestyle-adjusted relative risk <sup>a)</sup>
Stages I and II	0.631 (0.405–0.984) <sup>b)</sup>	0.564 (0.350–0.911)
Stage III	1.30 (0.716–2.36)	1.88 (0.785–4.54)
Total	0.769 (0.540–1.09)	0.775 (0.531–1.13)

a) Adjusted for intake frequency of coffee, black tea, soybean paste soup and other soybean products, nuts, fruits, green and yellow vegetables, seaweed, fish, meat, eggs, and milk; cigarette consumption and alcohol use; frequency of pregnancy, child birth, and abortion; body mass index, and age.

b) In parentheses, 95% confidence interval.

become much less significant after adjustment for these two clinical parameters, while improved survival by consumption of green tea would remain significant even after adjustment for clinical variables other than these two. In fact, improved survival became non-significant ( $P>0.3$ ) when clinical parameters (metastasized axillary lymph nodes, PgR, clinical staging, histological spread, use of chemotherapy, and blood vessel invasion) were included in the model. However, when metastasized axillary lymph nodes and PgR were removed from the model, the improvement of survival by green tea consumption remained significant ( $P<0.05$ ) even after adjustment for all other variables, resulting in decreased recurrence with relative risk of 0.640 (95% confidence interval, 0.408–0.998), for consumption of  $\geq 5$  cups vs.  $\leq 4$  cups per day. It should be noted that the influence of green tea on prognosis of breast cancer is much less than the influence of the potential prognostic factors mentioned above. No association was found between consumption of green tea and therapeutic parameters such as chemotherapy, radiotherapy, and use of tamoxifen.

## DISCUSSION

Green tea is attracting much attention as a promising natural agent for cancer prevention, in part due to its lack of toxic effects. We previously revealed the cancer-preventive effects of drinking green tea in a prospective cohort study among a Japanese general population.<sup>1)</sup> Furthermore, we conducted a Phase I trial using green tea extract in tablet form with 108 Japanese healthy volunteers for six months and found no severe adverse effects among them.<sup>2)</sup> On the other hand, it is also important to know whether green tea influences cancer phenotypes, when cancer develops in spite of the risk reduction generated by use of green tea, since green tea may exert multiple functions against carcinogenesis, including inhibition of tumor promotion, and also against cancer cells, including inhibition of growth, metastasis, and urokinase activity.

We therefore examined the association between consumption of green tea and clinical characteristics of breast cancer. Increased consumption of green tea was found to be associated with decreased numbers of metastasized axillary lymph nodes among premenopausal patients with stage I and II breast cancer and increased expression of PgR among postmenopausal ones. Since metastasis to axillary lymph nodes and expression of PgR are the two most reliable predictors of recurrence, the prognosis of these breast cancer patients was studied in terms of green tea consumption. We found that long-term consumption of  $\geq 5$  cups per day of green tea, i.e., prior to clinical cancer onset, is significantly associated with lower recurrence in stage I and II breast cancer: relative risk of recurrence is 0.564 (95% confidence interval, 0.350–0.911) after

adjustment for other lifestyle factors. Intake of black tea did not influence the prognosis of breast cancer, possibly due to the fact that Japanese drink much less black tea than green tea: 51 or 79% of the study patients consumed  $\leq 1$  cup or  $\leq 3$  cups per day, respectively. Besides the consumption of green tea prior to cancer onset, possible changes in lifestyle after surgery may also influence the prognosis of breast cancer. This possibility must be separately investigated to identify post-surgical epidemiologic factors which may influence the prognosis.

A possible bias associated with consumption of green tea is that those consuming much green tea may be health-conscious and thus frequently receive the breast cancer screening test. However, this seems to be unlikely because no statistically significant association between consumption of green tea and clinical staging was observed among the study patients; consumption of green tea was not associated with a health-oriented lifestyle in our prospective cohort study among a Japanese general population.<sup>1)</sup>

We do not have a plausible explanation of why consumption of green tea is associated with different clinical parameters between premenopausal and postmenopausal breast cancer. The influence of host factors such as estrogen and other growth factors (menopausal status, age, host experiences influencing hormone metabolism as epidemiologic variables) on breast carcinogenesis must be investigated further, since some chemopreventives may interact with these host factors and generate different responses in different subpopulations, as in the case of the synthetic chemopreventive *N*-(4-hydroxyphenyl)retinamide and circulating insulin-like growth factor I levels.<sup>22)</sup> This will be one of the targets in our parallel study on breast cancer using molecular biological tools.<sup>23, 24)</sup> We observed neither improvement in prognosis nor alteration of cancer phenotypes by increased consumption of green tea in stage III breast cancer, which recurred among 54% of patients in 4 years, indicating that the potential of green tea is not manifested when breast cancer is advanced. Changes in phenotypes of breast cancer along with its progression, including loss of estrogen dependence, are clinically well-known, in particular between stage II and stage III breast cancer (see Table I). Our results suggest that green tea can not influence the malignant progression of breast cancer in advanced stages.

In addition, we found that six patients contracted a second primary breast cancer during the follow-up period. Although the number of cases is insufficient for definitive analysis, it is of interest that five of these consumed  $\leq 4$  cups a day while the other drank  $\geq 5$  cups per day, which can be compared with 154 and 190 patients without recurrence consuming  $\leq 4$  cups and  $\geq 5$  cups per day, respectively. This suggests that increased consumption of green tea may inhibit second primary breast cancer ( $P=0.07$ ),

over and above the reduction in recurrence. However, our data cannot discriminate whether this resulted from drinking green tea prior to clinical cancer onset or from consumption after surgery, because consumption was possibly unchanged by experience of cancer. Again, further investigation is needed.

Given the potential implications of our epidemiological findings, the observed association of green tea with inhibition of micro-metastasis and increased expression of PgR in breast cancer needs to be confirmed by further investigation, including laboratory studies.

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