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ORIGINAL PAPER

Soy and tea intake on cervical cancer risk: the Singapore Chinese Health Study

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Abstract

Purpose Soy isoflavones and tea catechins have immunomodulating and chemopreventive properties relevant for cervical carcinogenesis; however, there are limited epidemiologic data on the relationship of soy and tea consumption with cervical cancer risk. The aim of our study was to examine effects of soy and tea intake on cervical cancer risk among Singapore Chinese women.

Methods The association between intake of soy and tea drinking and cervical cancer risk was investigated in a prospective, population-based cohort of 30,744 Chinese women in Singapore with an average 16.7 years of follow-up and 312 incident cervical cancer cases. Multivariable proportional hazard models were used to estimate hazard ratio (HR) and 95% confidence interval (CI) of cervical cancer associated with intake levels of soy and tea.

Results High intake of soy alone was associated with a statistically borderline significant 20% reduced risk of cervical cancer (HR 0.80, 95% CI 0.61, 1.05) while green tea alone was not (HR 0.97, 95% CI: 0.76, 1.22). In stratified analysis, high intake of soy was associated with a statistically significant decrease in cervical cancer risk among green tea drinkers (HR 0.43; 95% CI 0.28, 0.69) but not among non-drinkers of green tea. The difference in the soy-cervical cancer risk association between green tea drinkers and non-drinkers was statistically significant (p for interaction = 0.004). This inverse association between soy intake and cervical cancer risk remained after further adjustment for human papillomavirus serostatus. Black tea consumption was not associated with cervical cancer risk.

Conclusions These findings suggest that a protective effect of soy against cervical cancer development may depend on green tea constituents.

Keywords Black tea · Cervical cancer · Green tea · Soy · Soy isoflavone

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Abbreviations

BMI	Body mass index
CI	Confidence interval
CIS	Carcinoma in situ
EGCG	Epigallocatechin-3-gallate
HR	Hazard ratio
HPV	Human papillomavirus
HSV-2	Herpes simplex virus 2
OC	Oral contraceptive
OR	Odds ratio

Introduction

A primary cause of cervical cancer is persistent infection with human papillomavirus (HPV) at the transformation zone, a region where columnar epithelium of the cervix

changes into squamous epithelium [1, 2]. Cervical cancer incidence has been declining in most developed countries since the 1960s due to successful efforts in screening for and vaccinating against oncogenic HPV types (i.e., 16 and 18) [1, 3]. Despite these efforts, cervical cancer remains one of the most common gynecological cancers worldwide [4, 5]. In Singapore, cervical cancer is the third leading cause of cancer mortality among reproductive-aged women [6]. Although cervical cancer incidence rates have been decreasing in Singapore, resulting from a nationwide screening program, a recent trend analysis showed that women born after 1960 may be at increased risk for cervical cancer compared to earlier birth cohorts [1]. Given the approximate decade-long latency period between the beginning of persistent infection with HPV and the clinical manifestation of pre-malignant lesions, only a small proportion of these early low-grade lesions eventually progress to invasive cervical cancer in infected women [7]. Other factors, along with HPV, likely play an important role in the development of invasive cervical cancer.

Although cervical cancer is not considered a hormone-dependent malignancy, estrogen exposure in combination with persistent HPV infection is important in the development of cervical cancer. For example, epidemiological studies have identified multiparity and long-term oral contraceptive use as HPV cofactors associated with cervical cancer risk [8]. Evidence from virology- and immunology-based experimental studies implicates estrogen in cervical carcinogenesis through mechanisms including the initiation of cell proliferation and differentiation in the cervical transformation zone, local cervical immune microenvironment and cytokine-dependent immune response changes, and HPV gene expression [9]. Isoflavones are a plant-based estrogen (phytoestrogen) found most abundantly in soy products. As weak estrogens, soy isoflavones interact with estrogen receptors and have been shown to inhibit multiple estrogen-dependent and non-dependent cancers including breast, prostate, gastric, and lung cancers [10–13].

Green tea is a major source of epigallocatechin-3-gallate (EGCG) [14]. EGCG inhibits cell growth of HPV-infected cells by down-regulating E6 and E7 oncoproteins in vitro, and tumors regress when EGCG was combined with a DNA vaccine in tumor mouse model [15]. Other major tea catechins in green tea are epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC). Black tea contains only one-third to one-tenth catechins that green tea does, but has high levels of other tea polyphenols such as theaflavins, thearubigins, and gallic acid tannins [16, 17]. Given the different types of polyphenols and different concentrations of tea catechins, green, and black tea may have different effects on risk of cervical cancer. However, there have been no reports on prospective studies that have evaluated the relationship between green and black tea intake and cervical cancer risk.

The Singapore Chinese Health Study is a population-based, prospective cohort with detailed dietary intake information on a population with substantial intake of soy and green and black teas. The aim of this study is to investigate the direct and possible interactive effects of soy and tea intake on cervical cancer risk among Chinese women in Singapore. In a subanalysis for a nested case–control study of cervical cancer within the Singapore Chinese Health Study, we also evaluated whether HPV serological status modified the association between the intake of soy and tea and risk of cervical cancer.

Materials and methods

Participants

The details of the study design of the Singapore Chinese Health Study have been described previously in detail [18]. Briefly, cohort participants were recruited from permanent residents or citizens of Singapore, aged 45–74 years, living in government housing and belonging to one of the two major dialect groups (Hokkien and Cantonese). The study enrolled 35,303 women and 27,954 men from April 1993 to December 1998. All cohort participants completed a baseline in-person interview, including questions on demographics, lifestyle factors, and medical history. Questions specific for women obtained information on reproductive and menstrual history, hormone use, and history of cervical cancer screening (i.e., Pap-based test) from the full cohort.

The baseline interview also included a validated 165-item food-frequency questionnaire (FFQ) to assess usual dietary intake [19]. The FFQ measured the intake of the seven most common non-fermented soy products in the Singapore Chinese diet, including plain tofu, taukwa, taupok, foojook, foopei, tofu-far, and soybean drink, in eight predefined categories: never or hardly ever, once month, two to three times a week, four to six times a week, once a day, and two or more times a day. Soy intake was expressed based on equivalent amounts of tofu per day and grams of soy protein, and soy isoflavones, as previously described [20, 21]. Equivalent amounts of tofu per day was calculated using an algorithm that accounts for the varying water content across the seven soy foods. For example, since taukwa accounts for 89% of cooked plain tofu by weight and 69% of taukwa, we determined that 1 g of taukwa is equivalent to 2.8 g (31/11) of plain tofu [19]. Calculation of soy protein intake was based on the Singapore Food Composition Database [19]. The total soy intake and total soy protein is the sum of all soy foods expressed in units of plain tofu equivalent and the sum of the protein contents of soy foods, respectively. Market samples

of common soy foods in the Singapore diet were used to estimate the amount of genistein, daidzein, and glycitein soy isoflavones. The sum of these three isoflavones estimated the total soy isoflavone intake. Green tea and black tea drinking were assessed in the FFQ. Individuals indicated their drinking frequency for each tea separately based on the following categories: never or hardly ever, one to three times a month, once a week, two to three times a week, four to six times a week, once a day, two to three times a day, four to five times a day, or six or more times a day. Validation studies also demonstrated a strong correlation between self-reported tea and soy with urinary biomarkers of catechins and isoflavones, respectively [22, 23].

Biospecimens were collected from a 3% random sample of the entire cohort between 1994 and 1999. Beginning in 2000, we extended the collection of biospecimens to all surviving cohort participants. By April 2005, blood sample were obtained from 32,543 subjects, representing a 60% consent rate. Of the 52,322 eligible subjects, 28,346 subjects (54% of eligible) donated blood samples (designated as biospecimens subcohort). The components of blood including serum, plasma, buffy coats, and red blood cells were separated immediately after blood collection and stored at − 80 °C for future analysis.

Identification of cancer cases

Cancer diagnosis and deaths among cohort participants were identified through linkages with the Singapore Cancer Registry and the Singapore Registry of Births and Deaths, respectively. The national cancer registry has been in place since 1968 and is considered a comprehensive record of cancer cases in the country [24]. After excluding all men, women with baseline prevalent invasive cancer or intraepithelial (non-invasive) cervical cancer ($n = 1,276$), women who reported having a hysterectomy at baseline ($n = 3,278$), and women who were missing cervical cancer screening history ($n = 5$), 30,744 women remained and were followed. During follow-up, 312 women developed incident cervical cancer (first cancer) including 120 carcinoma in situ (CIS) and 192 invasive cervical cancer.

Nested case–control study

A nested case–control study was conducted within the cohort to evaluate HPV status as a potential confounder in the assessment of soy and tea associations with cervical cancer risk. There were 75 CIS or invasive cervical cancer cases and 439 non-cases with serum samples available for the present study. For each case, three controls were randomly selected among all eligible women without history of cancer or hysterectomy who were matched with the index case on age at study enrollment (within 3 years), dialect group (Hokkien, Cantonese), date of study enrollment (within 2 years), date of biospecimen collection (within 6 months), and baseline menopausal status (pre-, postmenopausal). We successfully identified three controls for each the of 52 cases, two controls for each of the 20 cases, and one control for each of the three cases; the total number of matched controls was 199.

Serologic testing

Antibody reactivities to HPV and herpes simplex virus 2 (HSV-2) antigens were examined as previously described at the German Cancer Research Center, Heidelberg, Germany [25]. Briefly, the Luminex® multiplex platform based on fluorescence-labeled polystyrene beads combined with glutathione S-transferase (GST) captures enzyme-linked immunosorbent assay (ELISA) was used to simultaneously test for 8 HPV types and HSV-2. The assay detected antibodies of the major capsid protein L1 of the high-risk mucosal HPV types 16, 18, 31, 33, 35, 45, 52, and 58. Serum antibodies against HSV-2 were also quantified. Reactivity of each HPV and HSV-2 antibody was quantified as Median Fluorescence Intensity (MFI), and antibody-specific cutoffs were defined to determine seropositivity for each type-specific capsid protein. Cutoffs were established based on a previously described study among 371 virgin Korean women with no evidence of genital HPV [26]. The cutoff was defined as five times the standard deviations above the mean of the final distribution of MFI values among these women after the exclusion of outliers. The Luminex® multiplex platform assay has high concordance with other HPV serology assays, and detects a larger proportion of weak antibody responses [25]. The case/control status of our test samples was blind to the laboratory personnel.

Statistical analyses

Proportional hazard regression models were used to estimate the cervical cancer risk, measured by hazard ratios (HR) and 95% confidence intervals (CI), associated with soy and tea intake, in addition to known cervical cancer risk factors such as lifestyle factors, reproductive health history, Pap screening history, and smoking history. Person-years of follow-up were calculated for each person from the date of the baseline interview to the date of first cancer diagnosis, death, out migration, or 31 December 2013, whichever occurred first. Daily intakes of soy products and soy isoflavones (genistein, daidzein, glycitein, and total) were expressed as weight/1,000 kcal to adjust for total energy and categorized by tertiles based on distribution among all women in the cohort. We categorized intake frequencies of green and black tea, separately, into non-drinker and drinker, and for drinkers, further grouped into monthly and weekly/

daily drinkers. All multivariable regression models included following potential confounders: dialect group (Cantonese, Hokkien), age (years), education level (no formal/primary education, and secondary or higher), and total calorie intake (kcal/day). We used a stepwise regression approach to determine other potential confounders that were found to be associated with risk of cervical cancer in the study population. According to an entry criterion of $p < 0.1$ and a removal criterion of $p < 0.2$ for a given variable, the following variables were included in the multivariable models: parity (zero to two, three to four, five or more births), duration of oral contraceptive (OC) use (never use, < 5 years, or ≥ 5 years), Pap screening history (never, ever), and menopausal status (premenopausal, postmenopausal). Adjustment for smoking status did not substantially alter the association between the exposure variables (i.e., soy and tea intake) and risk of cervical cancer, but was retained in the regression models to account for potential residual confounding. For linear trend test, ordinal values of soy and tea intake were used for the assessment of cervical cancer risk. To evaluate potential interaction between soy and tea intake on risk of cervical cancer, a product term of these two was created and included in multivariable models in addition to stratified analyses.

In analysis of the nested case–control dataset, conditional logistic regression models were used to assess the association between intake of soy and tea and risk of cervical cancer with additional adjustment for serology status of HPV and HSV-2. The latter was a proxy for sexual behavior. A positive serology status of HPV was determined if antibodies against the L1 protein was detected for any high-risk mucosal HPV types. All analyses were performed in SAS 9.3 (SAS Institute, Inc.). All p values are two sided. p values < 0.05 were considered statistically significant.

Results

The majority of the women were postmenopausal at baseline, with an average age of 56.2 years (SD = 8.0). The mean years of follow-up was 16.7 (SD = 4.3 years). The mean ages at diagnosis were 63.1, 60.4, and 64.8 years for total, CIS, and invasive cancer cases, respectively. Women with > 5 years of OC use had a statistically significant 62% increased risk of cervical cancer compared to non-OC users; women with two or less children had a statistically significant 58% reduced risk of cervical cancer compared with those with five or more children (Table 1). A history of Pap test was also associated with a 30% reduced risk of cervical cancer. High level of education was associated with reduced risk of cervical cancer. We observed no association for cervical cancer risk with cigarette smoking, physical activity, or menopausal status. Similar patterns of the risk associations were observed for patients with CIS and invasive cancer (data not shown) with one exception. History of Pap-based screening was associated with lower risk of invasive cervical cancer (HR 0.52; 95% CI 0.37, 0.74), but not with CIS (HR 1.01; 95% CI 0.69, 1.18).

The distributions of baseline characteristics varied across different levels of soy and green tea intake (Table 2). Women who consumed higher amounts of soy or green tea at least monthly (drinkers) were younger, premenopausal, less likely to smoke cigarettes, had a higher level of education, and a history of Pap-based test. Compared with those who did not drink green tea, green tea drinkers were more likely to also drink black tea. Green tea drinkers and black tea drinkers on average consumed 19.9 cups/month (SD = 30.4) and 15.9 cups/month (SD = 19.9), respectively.

High consumption of soy was associated with a statistically borderline significant 20% reduced risk of cervical cancer in all women (p value = 0.10) after adjustment for level of education, duration of OC use, history of Pap test, parity, menopausal status, and daily total calorie intake (Table 3). A weak inverse association for risk of cervical cancer was observed for total soy isoflavones (Table 3), as well as intake of individual specific soy isoflavones such as genistein, daisein, or glycitein (data not shown). Similar associations for soy and isoflavones were observed with risk of invasive cancer and CIS (data not shown).

Overall green tea consumption was not associated with cervical cancer risk (Table 4). In contrast, more frequent intake of black tea was associated with a weak positive association with risk of cervical cancer overall, but a stronger association was observed with risk of CIS (HR 1.56; 95% CI 1.04, 2.33, p for trend = 0.03). There was no association between intake of black tea and risk of invasive cancer (HR 1.08; 95% CI 0.76, 1.54, p for trend = 0.72). Results for frequent tea drinkers also showed no association with cervical cancer risk (see Supplemental Table S1).

We evaluated the potential modifying effects of tea intake on a soy-cervical cancer association (Table 5). Among green tea drinkers, highest tertile of soy intake was associated with a statistically significant 57% reduced risk of cervical cancer among green tea drinkers who drank at least monthly (p for trend < 0.001), whereas a null association among non-drinkers (p for trend = 0.52). The difference in the soy-cervical cancer risk association between green tea drinkers and non-drinkers was statistically significant (P for interaction = 0.004). Similarly, among green tea drinkers, the HRs (95% CIs) of invasive cervical cancer and CIS for third versus first tertile of soy intake were 0.40 (0.22, 0.73) and 0.48 (0.23, 0.98), respectively. The significant inverse association between soy intake and cervical cancer risk among green tea drinkers remained after excluding cancer cases and person-years of first 3 years of follow-up. Compared with the lowest tertile, the HRs (95% CIs) for the middle and highest tertiles of soy intake were 0.50 (0.29, 0.84) and 0.47 (0.27, 0.79),

Table 1 Selected baseline characteristics in relation to cervical cancer risk, the Singapore Chinese Health Study, 1993–2013 ($n = 30,744$)

	Cases, *n*	Person-years	HR (95% CI)[a]	*p* for trend
Characteristic				
Education level				
None/primary	267	405,734	1.00 (reference)	
≥ Secondary	45	108,040	0.59 (0.42, 0.82)	
Body mass index (kg/m^2)				0.148
< 24	209	359,372	1.00 (reference)	
24–< 28	72	114,792	1.08 (0.82, 1.41)	
≥ 28	31	39,609	1.34 (0.92, 1.95)	
Overall physical activity level[b]				0.754
None	240	385,640	1.00	
Low	50	95,429	0.85 (9.63, 1.15)	
Medium	17	24,679	1.10 (0.67, 1.80)	
High	5	8,025	0.99 (0.41, 2.41)	
Cigarette smoking				
Never	284	473,465	1.00 (reference)	
Ever	28	40,308	1.15 (0.78, 1.71)	
Parity				<0.001
≥ 5	101	137,705	1.00 (reference)	
3–4	143	193,732	0.87 (0.66, 1.16)	
0–2	68	182,336	0.42 (0.30, 0.60)	
Oral contraceptive use				0.067
Never used	220	375,283	1.00 (reference)	
≤ 5 years	60	104,781	0.97 (0.73, 1.3)	
> 5 years	32	33,709	1.62 (1.11, 2.35)	
Pap test history				
Never had a Pap	209	308,606	1.00 (reference)	
Had at least one Pap	103	205,167	0.70 (0.54, 0.90)	
Menopausal status				
Premenopausal	105	169,829	1.00 (reference)	
Postmenopausal	207	343,944	1.01 (0.73, 1.39)	

CI confidence interval, *HR* hazard ratio

[a]Hazard ratios were adjusted for age, dialect group, and interview year

[b]Low: moderate physical activity only (> 1/2 h/week); medium: strenuous–vigorous physical activity only (> 1/2 h/week); high: moderate and strenuous–vigorous physical activity (> 1 h/week)

respectively (p for trend = 0.005) among green tea drinkers only (p for interaction = 0.005). There was no evidence for effect modification by black tea on the soy-cervical cancer risk association (Table 5).

Characteristics of the 75 cervical cases and 199 matched controls in the nested case–control analysis are shown in Supplemental Table S1. The mean age at enrollment was 53 years (range 45–71 years). Women who developed cervical cancer were more likely to be HPV seropositive and have higher soy intake; however, frequency of green tea intake and HSV-2 seropositivity was similar among cases and controls (Supplemental Table S2). Women seropositive for high-risk HPV types had twofold higher odds of cervical cancer, compared to seronegative women (OR 2.07; 95% CI 1.18, 3.62). HPV seropositivity was not associated with cervical cancer (OR 1.08; 95% CI 0.57, 2.08). A statistically significant inverse association with soy intake for cervical cancer remained after further adjustment for HPV and HSV serological status (Table 6). Similar to the findings from the cohort analysis, there was no association between green tea or black tea intake and cervical cancer in this case–control analysis, regardless of adjustment for serological status of HPV. In this case–control analysis, a stronger inverse association was found between soy intake and cervical cancer risk among green tea drinkers than non-drinkers (Table 6). The strength of the association for third versus first tertile soy intake did not meaningfully change after adjusting for HPV and HSV serology status.

Table 2 Distribution of baseline demographic and lifestyle characteristics by levels of soy and green tea intake among women, the Singapore Chinese Health Study at baseline, 1993–2013 ($n = 30,744$)

	Tertiles (*T*) of Soy food intake			Green tea intake	
	*T*1	*T*2	*T*3	Non-drinkers	Drinkers
Number of women	10,229	10,274	10,241	19,351	11,393
Mean age (years) (SD)	57.5 (8.3)	55.9 (7.9)	55.3 (7.8)	56.6 (8.1)	55.7 (8.0)
Body mass index (kg/m^2) (%)					
< 20	15.5	15.1	14.2	15.6	13.7
20–< 24	54.9	55.6	54.7	56.3	53.1
24–< 28	21.6	22.1	23.0	20.8	24.8
≥ 28	8.0	7.2	8.2	7.4	8.5
Education, ≥ secondary level (%)	17.0	21.2	23.0	17.7	25.0
Smoking status, ever (%)	10.8	7.9	7.9	9.7	7.4
Parity, ≥ 5 births (%)	30.9	27.7	26.2	30.1	25.2
Pap-based test, never (%)	64.4	59.9	58.6	64.2	55.5
Menopausal status, postmenopausal (%)	72.9	67.8	65.7	70.5	65.9
Green tea, drinkers[a] (%)	30.9	38.3	41.9	–	–
Black tea, drinkers[a] (%)	24.1	30.3	33.5	23.6	38.9
Mean daily intake (SD)					
Total energy (kcal)	1,320 (440)	1,413 (473)	1,462 (499)	1,360 (458)	1,465 (496)
Soy food[b] (g/1000 kcal)	29.4 (12.7)	65.2 (10.5)	131.5 (51.6)	72.5 (53.3)	80.2 (51.1)
Soy protein (g/1,000 kcal)	0.6 (0.3)	1.4 (0.3)	2.7 (1.0)	1.5 (1.1)	1.7 (1.0)
Soy isoflavones (mg/1,000 kcal)	4.7 (2.3)	10.8 (2.5)	22.5 (10.2)	12.1 (9.8)	13.6 (9.4)

[a]Tea drinkers defined as those who drank tea at least monthly

[b]Expressed in units of tofu-equivalents

Table 3 Tertile levels of soy and soy isoflavone intake in relation to risk of cervical cancer, the Singapore Chinese Health Study, 1993–2013 ($n = 30,744$)

	Tertiles (T)			*p* for trend
	*T*1	*T*2	*T*3	
Soy food				
Cases, *n*	115	102	95	
Person-years	167,081	173,195	173,497	
Median (g/1000 kcal)	31.29	64.70	115.86	
HR (95% CI)[a]	1.00 (ref)	0.86 (0.66, 1.13)	0.80 (0.61, 1.05)	0.111
Total soy isoflavone				
Cases, *n*	111	101	100	
Person-years	166,821	173,445	173,507	
Median, mg/1000 kcal	4.85	10.60	19.63	
HR (95% CI)[a]	1.00 (ref)	0.89 (0.68, 1.16)	0.88 (0.67, 1.15)	0.347

CI confidence interval, *HR* hazard ratio, *T* tertile

[a]Hazard ratios were adjusted for age, dialect group, year of interview, level of education, smoking status, duration of oral contraceptive use, history of Pap-based test, parity, menopausal status, and daily total calorie intake

Discussion

Persistent infection of high-risk HPV types is a causal factor of cervical cancer [1]. Given that majority of women infected with HPV do not develop cervical cancer, infection with HPV alone is not a sufficient factor for the development of invasive cervical cancer [27]. Other factors, such as diet, may play a significant role in the progression of HPV-initiated cervical cancer [28, 29]. The traditional Asian diet is characterized by relatively high soy and green tea. Isoflavones and catechins found in soy

Table 4 Intake frequencies of green and black tea in relation to risk of cervical cancer, the Singapore Chinese Health Study, 1993–2013 ($n = 30,744$)

	Green tea			Black tea		
	Cases, *n*	Person-years	HR (95% CI)[a]	Cases, *n*	Person-years	HR (95% CI)[a]
Tea drinker						
Non-tea drinkers	200	323,141	1.00 (reference)[b]	211	360,748	1.00 (reference)[c]
Tea drinkers[d]	112	190,632	0.97 (0.76, 1.22)	101	154,025	1.19 (0.93, 1.51)
Intake frequency						
Non-tea drinkers	200	323,141	1.00 (reference)[b]	211	360,748	1.00 (reference)[c]
Monthly	34	60,459	0.92 (0.64, 1.32)	22	39,061	1.00 (0.64, 1.55)
Weekly/daily	78	130,173	0.99 (0.76, 1.29)	79	113,965	1.26 (0.96, 1.64)
p for trend			0.868			0.102

CI confidence interval, *HR* hazard ratio

[a]Hazard ratios were adjusted for age, dialect group, year of interview, level of education, smoking status, duration of oral contraceptive use, history of Pap-based test, parity, menopausal status, and daily total calorie intake

[b]The reference group included only non-green tea drinkers

[c]The reference group included only non-black tea drinkers

[d]Tea drinkers defined as those who drank tea at least monthly

Table 5 Tertile levels of soy intake in relation to risk of cervical cancer stratified by intake of green or black tea, the Singapore Chinese Health Study 1993–2013 ($n = 30,744$)

	Tertiles (*T*) of Soy Food (g/1,000 kcal)			*p* for trend	*p* for interaction
	*T*1	*T*2	*T*3		
Non-green tea drinkers					
Cases, *n*	68	68	64		
Person-years	115,324	106,772	101,045		
HR (95% CI)[a]	1.00 (reference)	1.12 (0.8, 1.57)	1.12 (0.79, 1.58)	0.515	
Green tea drinkers[b]					
Cases, *n*	47	34	31		
Person-years	51,757	66,423	72,452		
HR (95% CI)[a]	1.00 (reference)	0.53 (0.34, 0.83)	0.43 (0.28, 0.69)	< 0.001	0.004
Non-black tea drinkers					
Cases, *n*	83	64	64		
Person-years	126,055	120,141	114,552		
HR (95% CI)[a]	1.00 (reference)	0.83 (0.60, 1.15)	0.86 (0.62, 1.20)	0.369	
Black tea drinkers[b]					
Cases, *n*	32	38	31		
Person-years	41,206	53,053	58,946		
HR (95% CI)[a]	1.00 (reference)	0.90 (0.56, 1.45)	0.67 (0.41, 1.11)	0.111	0.528

CI confidence interval, *HR* hazard ratio, *T* tertile

[a]Hazard ratios were adjusted for age, dialect group, year of interview, level of education, smoking status, duration of oral contraceptive use, history of Pap-based test, parity, menopausal status, and daily total calorie intake

[b]Tea drinkers defined as those who drank tea at least monthly

foods and green tea, respectively, have chemopreventive, anti-estrogenic, and immune-modulating activities that may protect against infection-associated cancers including cervical cancer [15, 30–33]. Using the data of a prospective cohort of Chinese women in Singapore, we reported the association of traditional cervical cancer risk factors and tested the hypothesis that higher soy and green tea intake could be associated with lower risk of cervical cancer. We reported no statistically significant associations between either soy or green tea alone and risk of cervical cancer. However, green tea intake modified the soy-cervical cancer association. A statistically significant inverse association for high soy intake with lower risk of cervical

Table 6 Nested case–control analysis of soy and green tea intake in relation to cervical cancer with and without adjustment for human papillomavirus (HPV) serology status

	Cases ($n=75$)	Controls ($n=199$)	Multivariable-adjusted OR (95% CI)	HPV-adjusted OR (95% CI)[†]
Soy food				
1st Tertile (*T*1)	33	64	1.00 (reference)[a]	1.00 (reference)[b]
2nd Tertile (*T*2)	25	65	0.69 (0.35, 1.34)	0.72 (0.37, 1.42)
3rd Tertile (*T*3)	17	70	0.43 (0.21, 0.90)	0.43 (0.20, 0.92)
p for trend			0.025	0.030
Green tea				
Non-drinker	41	112	1.00 (reference)[a]	1.00 (reference)[b]
Drinker	34	87	0.96 (0.53, 1.74)	0.96 (0.52, 1.78)
Non-green tea drinker				
Soy food				
Low (*T*1 + *T*2)	31	76	1.00 (reference)[c]	1.00 (reference)[d]
High (*T*3)	10	36	0.66 (0.28, 1.58)	0.61 (0.25, 1.50)
Green tea drinker				
Soy food				
Low (*T*1 + *T*2)	27	53	1.00 (reference)[c]	1.00 (reference)[d]
High (*T*3)	7	34	0.20 (0.06, 0.65)	0.19 (0.06, 0.65)[e]

CI confidence interval, *HSV* herpes simplex virus, *HPV* human papillomavirus, *OR* odds ratio, *T* tertile

[a]Conditional odds ratios were adjusted for education level, smoking status, duration of oral contraceptive use, Pap-based test history, parity, and daily calorie intake

[b]Conditional odds ratios were adjusted for covariates listed in footnote (a), and HSV-2 and HPV seropositive status. HPV status was defined as seropositivity to L1 for any high-risk HPV (16, 18, 31, 33, 35, 45, 52, and 58)

[c]Unconditional odds ratios were adjusted for matching factors (i.e., age, dialect group, interview year, time between baseline interview, and biospecimen collection) and education level, smoking status, duration of oral contraceptive use, Pap test history, parity, menopausal status, and daily calorie intake

[d]Unconditional odds ratios were adjusted for covariates listed in footnote (c) and HSV-2 and HPV seropositive status. HPV status was defined as seropositivity to L1 for any high-risk HPV (16, 18, 31, 33, 35, 45, 52, and 58)

[e]*p* for interaction between green tea and soy intake = 0.316

cancer was present among women who consumed green tea, but not among those who did not drink green tea.

Experimental evidence from HPV transgenic mice provides insight on the potential role of estrogen in cervical cancer carcinogenesis [reviewed in: [34]], suggesting that estrogen contributes not only to the onset but to the growth and progression of cervical cancer [35]. As phytoestrogens, soy isoflavones may prevent the development of cervical cancer by blocking endogenous estrogen-stimulated pathways [36, 37]. Women who regularly consume green tea may have lower circulating estrogens, compared with those who do not drink green tea [38–40]. Thus, it is biologically plausible that the anti-estrogenic influence of soy and green tea are more apparent when both of them are examined jointly.

Few studies have examined the potential interactive effects of catechins and isoflavones on cancer development. In vivo experiments using the Nobel rat model showed a greater decrease in the number of precancerous lesions in the prostate treated with a combined dietary soy and green tea regimen than rats given soy or green tea alone [41]. In addition, the rats fed both soy and green tea catechins demonstrated suppression of nuclear factor-kappaB (NFκB) p50 binding activity and decreased expression of inflammatory cytokines. Not only is NFκB activity effected by E6 and E7, the primary oncoproteins for HPV-induced carcinogenesis, but NFκB is constant and active during human cervical cancer progression [42]. Both tea catechins and soy isoflavones are involved in pathways that block the activation of NFκB, either directly or indirectly [43, 44], thereby suppressing tumor progression and inducing apoptosis [reviewed in: [42]]. The NFκB pathway may represent a potential target that soy and green tea act on in a synergistic manner to prevent cervical carcinogenesis.

A limited number of case–control studies have evaluated the potential relationship between soy and green tea intake on cervical cancer risk. In a study among Chinese women, no association was observed between increasing weekly soy food intake and cervical cancer or high-grade precancerous lesions (CIN2/3) [45]. This study also reported a 45% statistically significant reduced odds of cervical cancer among green tea drinkers, compared to non-green tea drinkers, after adjusting for soy food intake and other dietary and lifestyle

habits. The authors did not report whether there were interactive effects of soy and green tea, perhaps because the study size was relatively small ($n = 104$ cases) [45]. In a case–control study among Hawaiians, levels of soy and green tea intake were similar between cervical squamous intraepithelial lesions (SIL) cases ($n = 122$) and controls ($n = 183$) [46]. Plasma levels of daidzein, glycitein, and genistein were also not associated with SIL, while a positive relationship with plasma equol was reported [46]. This association with equol, an intestinal bacterial metabolite of the soy isoflavone daidzein [37], was unexpected because equol and daidzein treatment inhibits cell growth in human cervical cancer HeLa cells and stimulates apoptotic cell death [47, 48]. Our finding for an inverse association with soy intake among green tea drinkers was not consistent with the case–control findings from the Chinese [45] or US population [46]. The use of a prospective study design and a validated FFQ limited the influence of bias due to differential misclassification of soy and green tea intake on our findings [19, 22], but differential misclassification by disease status could have contributed to null and positive associations reported in the two previous case–control studies. Our findings should be interpreted cautiously, because we cannot rule-out residual confounding due to correlated diet and/or lifestyle factors, nor can we discount the opportunity for spurious associations due to small numbers. However, these results do provide preliminary evidence that warrant future investigation into the potential effect of soy and green tea intake on cervical carcinogenesis.

Black tea consumption was not associated with cervical cancer risk in our cohort analysis including both invasive and CIS cases. However, we did observe a statistically significant increase in risk of CIS for weekly/daily intake versus none. A recent review of black tea and gynecologic cancers found that an increased risk of endometrial cancer and black tea intake (OR 1.20; 95% CI 1.05, 1.38) [15]. Based on a recent meta-analysis of prospective cohort studies, an estimated 4% increase in breast cancer risk was reported per cup of black tea consumed (RR 1.04; 95% CI 1.01, 1.08) [49]. In our Singapore cohort database, we reported an increase in prostate cancer risk for men who regularly drank black tea compared to non-drinkers (HR 1.41; 95% CI 1.03, 1.92) [50]. The potential adverse effect of black tea on hormone-related cancers may be related to higher levels of circulating estrogens associated with black tea intake [40]. In addition, the levels of catechins, the chemopreventive compounds in tea, are tenfold lower in black, compared with green tea [16]. Thus, it is not implausible that black and green tea have different or opposing associations with cancer risk. Our findings for a positive association between black tea and cervical CIS are certainly in line with results from studies of other hormone-related cancers.

The primary strength of our analysis was the prospective cohort design; we were able to ascertain soy and tea intake, as well as covariate exposure prior to cervical cancer diagnosis. Another strength of our study was the use of a validated FFQ that was designed for and validated in our study population [19, 22, 23].

The lack of information on the history of sexual activity among cohort participants was a limitation of our analysis. Sexual behavior is a known risk factor for cervical cancer and we were unable to assess whether the observed associations were independent of sexual behavior. However, the nested case–control analysis allowed the testing of serological markers for HPV and HSV, which are good proxy markers of sexual activity. HPV serology is a good indicator of pervious HPV exposure and a maker of persistent HPV infection; however, there is potential misclassification of HPV-infected women since not all women exposed to HPV will seroconvert [51, 52]. Compared with women in the entire cohort, the controls were somewhat younger (mean age = 54 vs. 56), were more likely to have had a Pap-based test (51% versus 39%), and consumed more soy (mean intake = 79 g/1,000 kcal/day versus 75 g/1,000 kcal/day). These differences may have contributed to the somewhat stronger, statistically significant inverse association between soy intake and cervical cancer risk in the case–control analysis, compared with the weak, non-significant inverse association in the cohort analysis. It is important to note, our study population's daily caloric intake is low, while green tea and soy consumption is high. Therefore, the findings in this study may not be generalizable to populations in western countries, where the dietary patterns are different from Singapore Chinese women or women from other Asian countries. For example, not only is the mean consumption of soy products (< 1 g/day) in European countries lower than in Asian countries, but the products consumed vary between the two regions [53]. However, unlike western countries, this study population is suitable for epidemiologic study of soy and cervical cancer associations.

In conclusion, high soy intake was associated with a statistically significant reduction in cervical cancer risk among Singapore Chinese women who were drinkers of green tea. These novel findings add important information to the existing literature which has a limited number of retrospective case–control studies that so far reported inconsistent results of soy and green tea intake in relation to cervical cancer risk. Future prospective epidemiologic studies are warranted to confirm our novel finding. In addition, experimental studies are needed to elucidate the mechanisms that underlie a potential combined effect of soy isoflavones and tea catechins on inhibiting cervical cancer progression. Even with current prevention strategies such as HPV vaccination and cervical cancer screening programs, further investigation of dietary factors is of public health interest.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

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