

The effect of the cyclin D1 (*CCND1*) A870G polymorphism on colorectal cancer risk is modified by glutathione-S-transferase polymorphisms and isothiocyanate intake in the Singapore Chinese Health Study

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Cyclin D1 (*CCND1*) regulates cellular decision between proliferation and growth arrest. Despite the functional relevance of the *CCND1* A870G single nucleotide polymorphism (SNP) published results on its association with colorectal cancer (CRC) were inconsistent. We examined the association between this *CCND1* genotype and CRC in the Singapore Chinese Health Study, a prospective investigation of diet and cancer in 63 000 Chinese men and women. We explored the hypothesis that inconsistency regarding the *CCND1*/CRC association may be attributable to the modifying effect of additional CRC risk factors. Since *GSTM1*/*GSTT1* genotype and dietary isothiocyanate (ITC) intake had previously been identified as CRC risk factors in this cohort, we now explored if they influenced the *CCND1*/CRC association. In a nested case-control study within the Singapore Cohort, genomic DNA collected from 300 incident CRC cases and 1169 controls was examined for *CCND1*, *GSTM1*, *GSTT1* and *GSTP1* polymorphisms. Unconditional logistic regression was used to assess genotype effects on cancer risk. No main effect of *CCND1* was observed, yet the *CCND1* effect was influenced by ITC intake and *GST* genotypes. The presence of at least one *CCND1* A-allele was associated with increased risk among low dietary ITC consumers (intake below median value for the cohort) with a high-activity *GST* profile (≥ 2 of the 3 *GST* genotypes classified non-null or high-activity) [odds ratio (OR) = 2.05; 95% confidence interval (CI), 1.10–3.82]. In contrast, the presence of at least one A-allele was associated with a decreased risk among all remaining subjects (OR = 0.56; 0.36–0.86) (P for interaction = 0.01). Recent studies indicate that ITCs inhibit cell proliferation and cause apoptosis through pro-oxidant properties. The results of our current study on CRC and those of our previous breast cancer study are compatible with the notion of oxidative stress in target cells as important determinant of direction and magnitude of the *CCND1* effect.

Abbreviations: *CCND1*, cyclin D1 gene; CRC, colorectal cancer; *GST*, glutathione-S-transferase; ITC, isothiocyanate; SNP, single nucleotide polymorphism.

Introduction

Cancer is viewed in part as a cell-cycle disease (1). The cyclin D1 protein, which is encoded by the *CCND1* gene located on chromosome 11q13, is therefore an important candidate gene in tumorigenesis and cancer progression. As a key cell-cycle regulatory protein modulating the restriction point early in the G₁ phase, cyclin D1 is a central determinant in the cellular decision between survival, proliferation and death (2–5).

For the cyclin D1 locus over 100 single nucleotide polymorphisms (SNPs), but no common missense SNP have been identified so far (6). The common A870G SNP remains the most frequently investigated and the only functionally characterized polymorphism. It modulates alternate splicing of *CCND1*. Both alleles lead to the expression of two different transcripts, but at different proportions. The A-allele as the major source of transcript form b may lead to a protein with a longer half-life, because it lacks a PEST sequence postulated to target for rapid degradation (7–10). Additional polymorphisms in *CCND1* or trans-acting elements may further modulate the splicing event (6).

Knudsen and colleagues (6) have recently reviewed the epidemiological evidence regarding the association between the *CCND1* A870G SNP and cancers at different sites. While the majority of studies linked the A-allele, especially in the state of A/A genotype to increased cancer risk and poor prognosis, some studies have also implicated the G-allele as risk allele or did not find a risk association at all. Published results on the associations between the *CCND1* SNP and colorectal cancer (CRC) are also mixed (reviewed in refs 6,10,12–19). The A/A or A/G genotype was associated with early disease onset in sporadic and hereditary cancer (15,16). Studies have reported either G/G (14) or A/A and A/G genotype (12,17,19,20) to be risk genotypes for CRC or adenomas. McKay *et al.* (18) found no association between the A870G SNP and CRC. Previous studies mostly lacked the inclusion of potential genetic/environmental effect modifiers of the *CCND1*/cancer association.

In the Singapore Chinese Health Study, a prospective investigation of diet and cancer in 63 000 Chinese men and women of Singapore, we examined the potential association between *CCND1* genotype and CRC risk. In addition, we explored the hypothesis, that the inconsistency in the main effect of *CCND1* genotype on CRC may partly be attributable to the modifying effects of certain CRC risk factors on this gene–risk association. Earlier, we had reported an inverse association between dietary isothiocyanate (ITC) and CRC, especially among subjects possessing the *GSTM1*/*GSTT1* null–null genotypes in the Singapore Chinese Health Study (21). Experimental data have shown ITC, the primary source of which is through dietary intake of cruciferous vegetables, to be an inhibitory agent in carcinogen induced colon carcinogenesis in animals (22,23). As an additional

exploratory analysis, we therefore investigated whether this previously identified CRC risk pattern consisting of dietary ITC and glutathione-*S*-transferase (*GST*) genotypes exerts any influence on the *CCND1* SNP-cancer risk association.

Materials and methods

Study population

The subjects were participants of the Singapore Chinese Health Study, a population-based, prospective investigation of diet and cancer risk (24). Briefly, between April 1993 and December 1998, we recruited 63 257 Chinese men and women from two major dialect groups in Singapore (Hokkien and Cantonese). The subjects were between the ages of 45 and 74 years and resided in government housing estates; at that time 86% of Singapore's population lived in such facilities. At recruitment, a face-to-face interview was conducted by a trained interviewer, using a structured questionnaire to elicit information on demographics, lifetime use of tobacco, physical activity, menstrual and reproductive history, medical history and family history of cancer. The questionnaire included a dietary component assessing usual intake pattern (including frequency and portion size) during the previous 12 months on 165 food and beverage items (24). The vegetable section included nine cruciferous vegetables commonly consumed by Chinese in Singapore (1). Average daily intake of roughly 100 nutrient and non-nutrient compounds, including dietary ITC, was computed for each study subject via linkage to the Singapore Food Composition Table. This semi-quantitative food frequency questionnaire was subsequently validated against a series of 24 h diet recalls (24). Average daily intake of ITC was expressed as $\mu\text{mol}/1000$ kcal in all analysis to adjust for total energy intake.

Between April 1994 and July 1999, we collected blood and urine specimens from a random 3% sample ($n = 1898$) of study enrollees ($n = 63\,257$). If the subject refused to donate blood, he/she was asked to donate buccal cells collected through the use of a modified 'mouthwash' protocol based on published methods (25). We collected blood ($n = 908$) or buccal cell ($n = 286$) samples from 678 female and 516 male subjects during April 1994–July 1999. The participation rate in the biospecimen subcohort of the study was 63%. Of these subjects, 13 developed CRC by April 30, 2002, the remaining 1181 subjects constituted the reference group for the present study. Demographic characteristics (sex, age at recruitment, dialect group, level of education) of the control subjects who donated blood or buccal cells were comparable to the whole cohort participants.

Case ascertainment

We identified incident CRCs through the population-based Singapore Cancer Registry (26). As of April 30, 2002, 592 incident CRCs (ICD C18–C20) had developed among cohort subjects. All incident cases of CRC were contacted as soon as they were identified by cohort staff for the donation of biospecimens. However, due to refusal or early death, biospecimen collection was successfully obtained only from 53% of all the cases. Thus, blood ($n = 228$) or buccal ($n = 84$) specimens were available from 312 of 592 cases of CRC. We manually reviewed the pathology report of each case to verify his/her histopathological diagnosis of CRC. Compared with those who had no formal education, a higher proportion of subjects with primary school or higher education donated biospecimens (56 versus 46%). More male (56%) than female (49%) cases and more Cantonese (57%) than Hokkien (49%) cases donated biospecimens. Otherwise, the average age at diagnosis of cancer was comparable between cases with and without biospecimens (65.4 versus 66.1 years).

Of the 312 cases with DNA specimens, we excluded 8 cases: carcinoma *in situ* (1), carcinoid (11) and unknown histologic type (1). So the present study included 304 cases of histologically confirmed CRC. The *CCND1* genotypes were non-informative on 12 controls and 4 cases. Therefore, the present analysis included 1169 participants without and 300 subjects with CRC. A total of 151 CRC patients with stage 1 or stage 2 disease were classified as localized while 139 patients with stage 3 or stage 4 disease were classified as advanced cancer. Ten cases had unknown stage due to insufficient information. There were 173 colon cancer cases (ICD codes C180–C189) and 127 rectum cancer cases (ICD code C199 and C209).

The study protocol was approved by the Institutional Review Boards of the University of Minnesota, the National University of Singapore and the University of Southern California. All participants gave written, informed consents at the time of recruitment and at the time of biospecimen collection, respectively.

Genotyping methods

DNA was purified from buffy coats of peripheral blood and buccal cell samples using a QIAamp 96 DNA Blood Kit (Qiagen, Valencia, CA). Genotyping for *CCND1*, *GSTM1*, *GSTT1* and *GSTP1* was performed using the fluorogenic 5'-nuclease assay (TaqMan Assay) (27).

The TaqMan assays were performed using a TaqMan PCR Core Reagent kit (Applied Biosystems Inc, Foster City, CA) according to manufacturer's instructions.

The *CCND1* polymorphism (A/G) was described previously (28). The oligonucleotide primers for amplification of the polymorphic region of *CCND1* were GC091for (5'-CCCCAACAACTTCCTGTCCTACTA-3') and GC091rev (5'-AGGCTGCCTGGGACATCA-3'). In addition, the fluorogenic oligonucleotide probes (TaqMan MGB Probes; ABI) used to detect each of the alleles were GC091F (5'-CCTCACTTACCGGGTCA-3') labelled with 6-FAM to detect the G allele and GC091V (5'-CCTCACTTACTGGGTCA-3') labelled with VIC to detect the A allele. PCR amplification using $\sim 10\text{ng}$ of genomic DNA was performed in a thermal cycler (MWG Biotech, High Point, NC) with an initial step of 95°C for 10 min followed by 50 cycles of 95°C for 25 s and 62°C for 1 min. The fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System and the results analyzed with Sequence Detection Software (ABI). Experimental samples were compared with 12 controls to identify the three genotypes at each locus (A/A, A/G and G/G). Any samples that were outside the parameters defined by the controls were identified as non-informative and were retested.

The oligonucleotide primers for amplification of the polymorphic region of *GSTP1* were GC070for (5'-CTGGTGGACATGGTGTAATG-3') and GC070rev (5'-TGCTCACACCATAGTTGGTGTAGATGA-3'). In addition, the fluorogenic MGB oligonucleotide probes used to detect each of the alleles were GC070F (5'-TGCAAATACGCTCTCCCT-3') labelled with 6-FAM and GC070V (5'-TGCAAATACATCTCCCT-3') labelled with VIC (Applied Biosystems). PCR amplification consisted in the same procedure described above for *CCND1*.

Genotyping of the *GSTT1* and *GSTM1* loci using the TaqMan assay consisted of separate assays for *GSTT1*, *GSTM1* and the albumin control gene. The oligonucleotide primers for amplification of the *GSTT1*, *GSTM1* and albumin genes were GC003for (5'-GTGCAACACCTCTGGAGAT-3') and GC003rev (5'-AGTCCTTGGCCTTCAGAATGA-3'), GC004 for (5'-CTTGGAGGAACCTCCGTGAAAAG-3') and GC004 rev (5'-TGGAACCTCCATAACACGTGA-3'), GC005 for (5'-CGATTTTCTTTTATAGGCAGTAGC-3') and GC005 rev (5'-TGGAAACTTCTGCAAACTCAGC-3'), respectively. Fluorescent oligonucleotide probes, for detection of PCR reaction products, were synthesized to contain the dye 6-FAM (BioSearch Technologies, Novato, CA). The probes for the *GSTT1*, *GSTM1* and albumin genes were GC003FAM (5'-ATGCTGCCATCCCTGCC-3'), GC004FAM (5'-AAGCGGCCATGGTTTGCAGG-3') and GC005FAM (5'-CGCCTGAGCCAGAGATTTCCTCA-3'), respectively. PCR amplification using ~ 10 ng of genomic DNA was performed in an ABI 7900HT Sequence Detection System (Applied Biosystem) with an initial step of 95°C for 10 min followed by 50 cycles of 95°C for 25 s and 62°C for 1 min. The fluorescence profile of each well was measured in real-time during the PCR amplification and the results analyzed with Sequence Detection Software (ABI). Any sample with a fluorescence signal that crossed a threshold of 0.2 ΔR_n before cycle 40 was considered positive for the loci analyzed. Samples negative for both *GSTT1* and *GSTM1* must be positive for albumin to be called; otherwise, the sample was designated non-informative and retested. All analyses were carried out blind to case or control status.

Statistical analysis

Although we sampled our controls randomly from the whole cohort, our study is more case–control than case–cohort in design since time of follow-up was relatively short (mean 4.1 years; ranging from 6 days to 8.8 years) and comparable between cases and the subcohort, with only 13 subjects in the latter group developing CRC during the observation period. Therefore, we analysed our data using both case–cohort (Epicure software PEANUTS procedure, Hirosoft, Seattle, WA) and case–control methods (29) and the two sets of results are similar. Figures presented in this report are derived from our case–control analyses. Specifically, we used unconditional logistic regression methods to examine the effect of the A870G polymorphism of *CCND1* on CRC risk, with and without stratification by levels of dietary ITC and *GST* genotypes, separately and in combination. Indicator variables for the genotypes of *CCND1* (GG, GA, AA; GA/AA) were created using the GG-genotype as the reference category. The median value of dietary ITC (5.16 $\mu\text{mol}/1000$ kcal) among all cohort members was used to separate individuals into the high (above median) versus low (below median) intake categories. *GSTM1* and *GSTT1* null genotypes were subjects homozygous for the respective gene deletions. The *GSTP1* polymorphism has been shown to

influence GSTP1 activity, with the direction of the effect being substrate dependent (30). Based on prior empirical findings with cancer risk (31) *GSTP1* AA genotype was considered the putative high-activity genotype compared with the AB/BB genotypes in this current study. The strength of the gene–cancer associations was measured by odds ratio (OR) and its 95% confidence interval (CI). The following covariates were included in all gene–disease regression models: gender, age at recruitment (years), year of recruitment (1993–1998), dialect group (Hokkien, Cantonese), level of education (no formal education, primary school only, secondary school or higher), body mass index (BMI) (<20, 20 to <24, 24 to <28, or ≥28 kg/m²), smoking status at baseline (never, former, current), alcohol consumption at

baseline (none, <7, ≥7 drinks/week) and family history of CRC (yes, no). In all analyses, we formally tested for the potential difference in findings between men and women. Since no gender effects were found, all results are shown for both sexes combined. The polytomous logistic regression method was used to examine associations of interest by subsite (colon, rectum) and by stage of disease (localized, advanced) (32). The linear trend tests for exposure–disease associations (Table I) were based on ordinal values of the specific exposures.

Statistical analysis was carried out using the SAS version 9.1 (SAS Institute, Cary, NC). All *P*-values are two-sided and *P* < 0.05 were considered statistically significant.

Table I. Distribution of selected variables in CRC patients and controls, Singapore Chinese Health Study

Variables	Controls		Cases		OR* (95% CI)
	<i>N</i>	(%)	<i>N</i>	(%)	
Gender					
Male	504	(43)	172	(57)	—
Female	665	(57)	128	(43)	
Dialect group					
Cantonese	566	(48)	135	(45)	—
Hokkien	603	(52)	165	(55)	
Education					
No formal education	314	(27)	91	(30)	—
Primary school	504	(43)	149	(50)	
Secondary school or higher	351	(30)	60	(20)	
BMI (kg/m ²)					
<20	186	(16)	45	(15)	1.00
20–<24	653	(56)	144	(48)	0.78 (0.53–1.15)
24–<28	266	(23)	91	(30)	1.35 (0.88–2.07)
≥28	64	(5)	20	(7)	1.40 (0.75–2.64)
<i>P</i> -trend					0.01
Hours per week of moderate activities ^a					
<0.5	877	(75)	224	(74)	1.00
0.5–3	186	(16)	44	(15)	0.78 (0.53–1.14)
4+	106	(9)	32	(11)	0.82 (0.52–1.28)
<i>P</i> -trend					0.21
Weekly vigorous work/sports ^b					
No	1024	(88)	270	(90)	1.00
Yes	145	(12)	30	(10)	1.14 (0.73–1.79)
Cigarette smoking status at baseline					
Never	848	(73)	179	(59)	1.00
Ex	129	(11)	50	(17)	1.04 (0.68–1.59)
Current	192	(16)	71	(24)	1.22 (0.85–1.76)
Alcohol drinking at baseline (number of drinks/week)					
0	957	(82)	233	(78)	1.00
<7	164	(14)	42	(14)	1.00 (0.67–1.50)
7+	48	(4)	25	(8)	2.07 (1.20–3.59)
<i>P</i> -trend					0.04
Family history of CRC					
No	1141	(98)	287	(96)	1.00
Yes	28	(2)	13	(4)	2.36 (1.15–4.84)
Dietary ITC					
Low ^c	587	(50)	176	(59)	1.00
High	582	(50)	124	(41)	0.82 (0.63–1.08)
GSTM1 genotype					
Positive	643	(55)	168	(56)	1.00
Null–null	525	(45)	132	(44)	0.89 (0.68–1.16)
GSTT1 genotype					
Positive	693	(59)	200	(67)	1.00
Null–null	475	(41)	100	(33)	0.71 (0.54–0.94)
GSTP1 genotype					
AA ^d	770	(66)	211	(70)	1.00
AB	346	(30)	80	(27)	0.91 (0.67–1.23)
BB	53	(4)	9	(3)	0.68 (0.32–1.44)

*ORs were adjusted for gender, dialect group (Cantonese, Hokkien), age at recruitment (years), year of recruitment (1993–1998) and education (no formal education, primary school, secondary school or higher).

^aModerate activities included brisk walking, bowling, bicycling on level ground, tai chi or chi kung.

^bVigorous work/strenuous sports included moving heavy furniture, loading or unloading trucks, shovelling or equivalent manual labor, jogging, bicycling on hills, tennis, squash, swimming laps or aerobics.

^cLow = below median value (5.16 μmol/1000 kcal) for the entire cohort.

^dAA = putative high-activity genotype.

Results

The characteristics of the study population are summarized in Table II. Factors positively associated with risk were high BMI, cigarette smoking and alcohol intake. Dietary ITC was inversely associated with risk (P for trend with ITC intake as continuous variable = 0.19). Mean age at cancer diagnosis among the cases was 65.3 years [standard deviation (SD) = 7.7]. Mean age at recruitment was 61.2 years (SD = 7.5) for cases and 56.5 years (SD = 8.1) for controls.

The distribution of *CCND1* genotypes by case-control status and stage of disease is summarized in Table II. The frequency of the A-allele in control subjects was 59%. The genotype distribution among controls was in Hardy-Weinberg equilibrium (P = 0.27). Overall, there was no association between *CCND1* genotype and CRC. This genotype-cancer association did not differ by stage of disease or by subsite (colon versus rectum; data not shown).

Table II shows the associations between *CCND1* genotype and CRC risk separately for subjects with low versus high dietary ITC. Among subjects with high dietary ITC intake, the presence of one or two A-alleles was associated with a decreased risk of CRC (OR = 0.57; 95% CI = 0.33–0.97 and OR = 0.55; 95% CI = 0.31–0.96, respectively). The effect modification of the *CCND1*/CRC association by ITC intake level was not materially altered by shifting the ITC high versus low cutoff point (top quartile versus rest; lowest quartile versus rest; tertiles). The P -value for an interaction term between *CCND1* genotype (GG versus non-GG) and ITC intake as a continuous variable was 0.02.

Table II also presents the effects of *CCND1* genotype on CRC risk according to subjects' *GST* genotypes. Among subjects possessing the *GST* null (low- activity) genotypes, there was a consistent, inverse relationship between risk for

CRC and one or two copies of the *CCND1* A-allele relative to the GG-genotype. The OR was 0.60 (95% CI = 0.24–1.48) among subjects with the homozygous *GSTM1* and *T1* null-genotypes while the comparable figures for those with either the *GSTM1* or *T1* positive genotypes was 0.96 (95% CI = 0.66–1.40). The OR was 0.62 (95% CI = 0.34–1.13) among subjects with the *GSTP1* AB/BB (putative low-activity) genotypes while the comparable figures for those with the *GSTP1* AA genotype was 1.08 (95% CI, 0.70–1.66). When subjects were classified according to their combined *GSTM1/GSTT1/GSTP1* genotypes, the OR was 0.53 (95% CI = 0.28–0.98) among those with at most one of the three genotypes being classified as positive or high-activity (referred to as the low-activity *GST* profile). The comparable figures for subjects with at least two positive/high-activity genotypes out of the possible three (the high-activity *GST* profile) were 1.14 (95% CI = 0.75–1.74). None of the results presented in Table II were materially altered by excluding cases with <2 years of follow-up time from the analysis.

Table III presents the *CCND1* genotype/CRC risk associations according to subjects' combined status on dietary ITC and *GST* genotypes. The presence of at least one *CCND1* A-allele was associated with a significant increased risk for CRC among low consumers of dietary ITC who possessed a high-activity *GST* profile (OR = 2.05; 95% CI = 1.10–3.82). In contrast, the presence of at least one A-allele was associated with a significant decreased risk for CRC among all remaining subjects (i.e. those with either high dietary ITC levels or a low-activity *GST* profile) (OR = 0.56; 95% CI = 0.36–0.86). These two ORs were statistically significantly different from each other (P for interaction = 0.01). Again, the effect modification of the *CCND1*/CRC association by dietary ITC and *GST* genotypes was not materially altered by shifting the ITC high versus low cutoff

Table II. Cyclin D1 (*CCND1*) G870A genotype in relation to CRC risk, stratified by stage of disease, ITC intake and *GST* genotypes, the Singapore Chinese Health Study

Variables	CCND1 genotype							
	GG		GA		AA		GA/AA	
	Ca/Co	OR	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)
Total subjects	56/207	1.00	132/548	0.86 (0.59–1.25)	112/414	0.95 (0.65–1.39)	244/962	0.90 (0.64–1.27)
Localized ^a	31/207	1.00	59/548	0.67 (0.41–1.09)	61/414	0.91 (0.56–1.49)	120/962	0.78 (0.50–1.21)
Advanced	23/207	1.00	69/548	1.13 (0.68–1.88)	47/414	0.99 (0.58–1.69)	116/962	1.07 (0.66–1.73)
Low ITC ^b	25/103	1.00	80/273	1.27 (0.75–2.16)	71/211	1.54 (0.90–2.65)	151/484	1.39 (0.84–2.28)
High ITC	31/104	1.00	52/275	0.57 (0.33–0.97)	41/203	0.55 (0.31–0.96)	93/478	0.56 (0.34–0.91)
<i>GSTM1</i> positive (1)	35/131	1.00	66/293	0.80 (0.50–1.30)	67/219	1.12 (0.69–1.82)	133/512	0.94 (0.60–1.46)
<i>GSTM1</i> Null-Null (0)	21/76	1.00	66/254	0.89 (0.49–1.62)	45/195	0.75 (0.40–1.39)	111/449	0.83 (0.47–1.46)
<i>GSTT1</i> positive (1)	37/122	1.00	91/318	0.88 (0.56–1.40)	72/253	0.84 (0.52–1.36)	163/571	0.87 (0.56–1.33)
<i>GSTT1</i> Null-Null (0)	19/85	1.00	41/229	0.83 (0.43–1.58)	40/161	1.21 (0.63–2.32)	81/390	0.98 (0.54–1.78)
<i>GSTP1</i> AA (1)	35/134	1.00	96/351	1.09 (0.69–1.73)	80/285	1.06 (0.66–1.71)	176/636	1.08 (0.70–1.66)
<i>GSTP1</i> AB/BB (0)	21/73	1.00	36/197	0.52 (0.27–1.00)	32/129	0.78 (0.40–1.52)	68/326	0.62 (0.34–1.13)
<i>GSTM1</i> or <i>T1</i> positive	47/172	1.00	112/438	0.91 (0.61–1.37)	96/336	1.01 (0.67–1.53)	208/774	0.96 (0.66–1.40)
<i>GSTM1</i> and <i>T1</i> null	9/35	1.00	20/109	0.60 (0.23–1.59)	16/78	0.60 (0.23–1.59)	36/187	0.60 (0.24–1.48)
Summed score of <i>GSTM1/T1/P1</i>								
2,3 ^c	36/143	1.00	96/340	1.11 (0.71–1.74)	81/262	1.19 (0.74–1.89)	177/602	1.14 (0.75–1.74)
0,1 ^d	20/64	1.00	36/207	0.48 (0.24–0.95)	31/152	0.58 (0.29–1.17)	67/359	0.53 (0.28–0.98)

^aLocalized disease was defined as stages 1 and 2. Advanced disease was defined as stages 3 and 4.

^bBelow versus above median value for the entire cohort.

^cAt least 2 of the 3 *GST* genotypes being classified as positive or high-activity (referred as the high-activity *GST* profile).

^dAt most 1 of the 3 *GST* genotypes being classified as positive or high-activity (referred as the low-activity *GST* profile).

ORs were adjusted for gender, age at recruitment (years), year of recruitment (1993–1998), dialect group (Cantonese, Hokkien), education (no formal education, primary school, secondary or higher), BMI (<20, 20–24, 24–28, 28+ kg/m²), smoking status at baseline (never, former, current), alcohol consumption at baseline (none, <7, 7+ drinks/week) and family history of CRC (no, yes).

Table III. Cyclin D1 (*CCND1*) G870A genotype in relation to CRC risk, stratified by GST genotypes and ITC intake in combination, the Singapore Chinese Health Study

ITC ^a	Summed score of GST M1/T1/P1	CCND1 genotype							
		GG		GA		AA		GA/AA	
		Ca/Co	OR	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)
Low	2,3 ^b	15/77	1.00	61/172	1.96 (1.02–3.78)	53/142	2.17 (1.11–4.22)	114/314	2.05 (1.10–3.82)
Low	0,1 ^c	10/26	1.00	19/101	0.46 (0.17–1.25)	18/69	0.75 (0.27–2.08)	37/170	0.57 (0.23–1.43)
High	2,3 ^b	21/66	1.00	35/168	0.60 (0.31–1.15)	28/120	0.60 (0.30–1.20)	63/288	0.60 (0.33–1.10)
High	0,1 ^c	10/38	1.00	17/106	0.55 (0.20–1.48)	13/83	0.49 (0.18–1.35)	30/189	0.52 (0.21–1.29)
Low 0,1 or High 0,1,2,3		41/130	1.00	71/135	0.54 (0.34–0.85)	59/272	0.59 (0.36–0.96)	130/647	0.56 (0.36–0.86)

^aBelow versus above median value for the entire cohort.

^bAt least 2 of the 3 GST genotypes being classified as positive or high-activity (referred as the high-activity GST profile).

^cAt most 1 of the 3 GST genotypes being classified as positive or high-activity (referred as the low-activity GST profile).

ORs were adjusted for gender, age at recruitment (years), year of recruitment (1993–1998), dialect group (Cantonese, Hokkien), education (no formal education, primary school, secondary or higher), BMI (<20, 20–<24, 24–<28, 28+ kg/m²), smoking status at baseline (never, former, current), alcohol consumption at baseline (none, <7, 7+ drinks/week) and family history of CRC (no, yes).

point (top quartile versus rest; lowest quartile versus rest; tertiles).

Discussion

In the present study there was no main effect of *CCND1* A870G on CRC risk, but our exploratory analysis found that the *CCND1* A870G effect is modified by the level of ITC intake, *GST* genotype and their combination. The presence of an A-allele conferred an increased risk for CRC among subjects with low ITC intake and no *GST* deficiency. In contrast among subjects with either high ITC intake or a low-activity *GST* profile, the presence of an A-allele was associated with a decrease in CRC risk.

Our current findings relating *CCND1* genotype to CRC risk and those of our previous study on breast cancer in this same cohort (11) are suggestive of oxidative stress as the biological underpinning for the observed effect modification by *GST* genotypes, dietary *n*-3 and *n*-6 fats (breast cancer) and dietary ITC (CRC). Our hypothesis is based on several lines of evidence. First, cell-cycle checkpoint responses to oxidative stress, a significant source of endogenous and exogenous DNA damage, are well documented and include up- and down-regulation of cyclin D1. Experimental data demonstrate that reactive oxygen species (ROS) modulate the G₁ checkpoint and the direction of cyclin D1/Cdk4/6 activity on the cell cycle (33). Partially through the regulation of cyclin D1 expression, oxidants are important regulators of the signalling events dictating decisions between cell survival, proliferation or death (4,34,35). Second, novel evidence points to the relevance of this pathway to colorectal tumorigenesis. Malfunction of the Wnt signalling pathway with abnormal stabilization of β -catenin is a key causative step in CRC, and the effect may in part be attributed to an abnormal regulation of the oxidative stress impact on the cell cycle (36). β -catenin was identified as an important regulator of cyclin D1 in colon carcinoma cells (37). In addition, β -catenin was recently found to act as a cofactor for the FOXO subfamily of winged helix transcription factors, which induce cell-cycle arrest and quiescence in response to oxidative stress (36). Third, experimental evidence documents a pro-oxidative effect of ITCs on the cell-cycle and on

CCND1 expression. The biological mechanisms underlying the cancer-protective effect of ITC are still poorly understood (38). Initially the anticancer effects of ITC were attributed exclusively to modulation of carcinogen metabolism including the induction of oxidant-scavenging GSTs. More recent studies have indicated that ITCs can additionally inhibit proliferation of cancer cells by causing cell-cycle arrest and/or apoptosis (35,38,39–41). The growth inhibiting impact of ITCs on target cells is attributed to a pro-oxidant action of these compounds on cell-cycle progression/arrest and on cyclin D1 expression (35,39,42–46). For example, incubation of pancreatic cancer cell lines with *N*-acetyl-L-cysteine was associated with resistance to apoptosis induced by the ITC compound sulforaphane (47). In addition, sulforaphane was recently found to inhibit the serum-stimulated growth of the human colon cancer cell line HT-29 by blocking the cell cycle at G₁ phase via activation of MAPKs pathways, including ERK, JNK and p38. This effect was abrogated when the cell lines were exposed to both sulforaphane and an antioxidant simultaneously (38).

Our results are consistent with the laboratory data described above, and suggest that the A-allele protects against CRC among subjects with an enhanced state of oxidative stress (i.e. high ITC consumption or low-activity *GST* profile). These results are compatible with our previous report on the *CCND1* SNP association with breast cancer, in which the protective effect of the *CCND1* A-allele on breast cancer risk was mainly confined to women possessing a genetic/dietary profile that is associated with an enhanced state of oxidative stress (11). Interestingly, we now found that the A-allele increased CRC risk among subjects with a decreased state of oxidative stress (i.e. low ITC consumption and high-activity *GST* profile). This risk increasing effect of the A-allele under conditions of low oxidative stress was not statistically significant in our earlier breast cancer report (11). Previously, we demonstrated a protective effect of ITC intake on CRC (21) and colorectal adenomas (48) that was restricted to the *GSTM1* and *GSTT1* null genotypes. This observation was attributed to the effects of the GSTs on ITC excretion. In light of our present results, we may now also hypothesize that subjects with low intake level of ITC and functional *GST* enzymes are left with low levels of pro-oxidative acting ITC at a cellular level. An oxidant-level dependent direction

of the A-allele effect agrees well with the experimentally documented context-dependent dual role of cyclin D1, which also has been documented for other cell-cycle regulators (5,49–55). Functional data for the *CCND1* A870G SNP suggest that carriers of one or two A-alleles produce protein with a longer half-life (7,28,56–57). Our findings suggest that this gene variant increases protein activity on both cellular proliferation and growth inhibition, with the direction of the effect determined in part by the level of oxidative stress in target cells.

The present study is the first to investigate the association between the A870G *CCND1* SNP and CRC risk stratified by GST genotypes and ITC consumption. Results on the main effect of *CCND1* genotype on risk, prognosis and age at onset of familial and non-familial CRC have been inconsistent (6,10,13,15–19). The Hawaii/Los Angeles Multiethnic Cohort Study found the A-allele to be more prominent in CRCs diagnosed at a regional or distant stage (12). The associations were statistically significant in White and Hawaiian, but not in Japanese participants. In contrast, a case–control study in Singapore found the GG-genotype to relate to increasing risk (14). In the Hawaii/Los Angeles Multiethnic Cohort Study, mean daily intake of cruciferous vegetables among Japanese-American, White and Native Hawaiian participants were 44.9, 40.4 and 46.4 g/day, respectively, in men and 49.2, 43.2 and 51.4 g/day, respectively, in women (58). In the Singapore Chinese Health Study, average daily intake of cruciferous vegetables among cohort participants was 41 g/day (59). Therefore, there is no evidence that the possibly modifying effect of dietary ITC on the *CCND1* genotype–CRC association is behind the discrepant results noted in the US versus Singapore Chinese. Additional external as well as internal factors potentially underlying the disparate effects of the *CCND1* A870G SNP in different populations must be investigated. Smoking, obesity, family history of cancer and post-menopausal hormone use in women have been suggested as potential effect modifiers of *CCND1* A/G870 SNP/cancer association (20,60–61). Furthermore, modifiers including additional polymorphisms in the *CCND1* gene or *trans*-acting factors which influence transcript ‘a’ versus ‘b’ splicing of the *CCND1* gene independent of the A870G SNP must be identified (6). The latter aspect is of special relevance given that Bala and Ptelomaki (10) found *CCND1* transcript ‘b’ production, but not the A870G SNP to be associated with the prognosis of CRC.

This study has several strengths. Dietary assessment was conducted using a validated, semi-quantitative food frequency questionnaire. We had previously reported a highly statistically significant correlation between daily intake of cruciferous vegetables or daily ITC intake and urinary ITC levels in study subjects (59). All exposure assessments occurred prior to cancer diagnosis, and therefore, could be presumed to be free of recall bias. In addition, the study population is genetically homogeneous since they are full-blooded descendants of natives from two contiguous prefectures in southern China. There is a theoretical concern for selection bias arising from the higher availability of blood specimens among Cantonese versus Hokkien cases with CRC and the higher levels of education among cases with blood specimens versus those without blood specimens. We examined if *CCND1* genotype is related to dialect group or education among CRC cases, and no associations were found

($P = 0.68$ for dialect group; and $P = 0.89$ for education). Given that dialect group and education are not confounders of the hypothesized *CCND1*/CRC association in our study population, the unequal distributions of these two factors between cases with and without blood specimens should not impact on the validity of our study findings.

A major limitation of our study is its low statistical power for the assessment of gene–environment and gene–gene interactions, due to the rather modest number of incident CRCs to date within this cohort of Singapore Chinese subjects. However, the consistency of the results across the two different types of cancer (breast and colorectum) in this population-based cohort lends credence to the validity of both sets of results (11). As this long-term, prospective investigation continues to accrue incident cases of CRC, we will have the opportunity to revisit this hypothesis with sufficient statistical power, using a haplotype approach as opposed to examining single polymorphisms one at a time, and studying additional markers of oxidative stress.

The present study highlights the modifying effects of genetic and environmental cofactors on the low penetrance genotype in relation to a given disease risk. Thus, one should be cautious in generalizing a given genotype–disease association across populations with varying lifestyles, dietary habits or genetic background. This may be especially relevant for genes involved in tightly regulated processes such as cell cycle and DNA repair. Our exploratory findings suggest that the oxidant–antioxidant balance in cells may be an important determinant of the direction of the cyclin D1 checkpoint function and thus of the *CCND1* genotype effect on carcinogenesis.

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References

- Sherr,C.J. (1996) Cancer cell cycles. *Science*, **274**, 1672–1677.
- Malumbres,M. and Barbacid,M. (2001) To cycle or not to cycle: a critical decision in cancer. *Nat. Rev. Cancer*, **1**, 222–231.
- Ortega,S., Malumbres,M. and Barbacid,M. (2002) Cyclin D-dependent kinases, INK4 inhibitors and cancer. *Biochim. Biophys. Acta*, **1602**, 73–87.
- Cook,S.J., Balmanno,K., Garner,A., Millar,T., Taverner,C. and Todd,C. (2002) Regulation of cell cycle re-entry by growth, survival and stress signalling pathways. *Biochem. Soc. Trans.*, **28**, 233–240.
- Hunter,T. and Pines,J. (1994) Cyclins and cancer. II: cyclin D and CDK inhibitors come of age. *Cell*, **79**, 573–582.
- Knudsen,K.E., Diehl,J. A., Haiman,C.A. and Knudsen,E.S. (2006) Cyclin D1: polymorphism, aberrant splicing and cancer risk. *Oncogene*, **25**, 1620–1628.
- Betticher,D.C., Thatcher,N., Altermatt,H.J., Hoban,P., Ryder,W.D. and Heighway,J. (1995) Alternate splicing produces a novel cyclin D1 transcript. *Oncogene*, **11**, 1005–1011.
- Holley,S.L., Parkes,G., Matthias,C., Bockmuhl,U., Jahnke,V., Leder,K., Strange,R.C., Fryer,A.A. and Hoban,P.R. (2001) Cyclin D1 polymorphism and expression in patients with squamous cell carcinoma of the head and neck. *Am. J. Pathol.*, **159**, 1917–1924.

9. Howe, D. and Lynas, C. (2001) The cyclin D1 alternative transcripts [a] and [b] are expressed in normal and malignant lymphocytes and their relative levels are influenced by the polymorphism at codon 241. *Haematologica*, **86**, 563–569.
10. Bala, S. and Peltomaki, P. (2001) Cyclin D1 as a genetic modifier of hereditary nonpolyposis colorectal cancer. *Cancer Res.*, **61**, 6042–6045.
11. Ceschi, M., Sun, C.-L., Van den Berg, D., Koh, W.-P., Yu, M.C. and Probst-Hensch, N.M. (2005) The effect of cyclin D1 (CCND1) G870A-polymorphism on breast cancer risk is modified by oxidative stress among Chinese women in Singapore. *Carcinogenesis*, **26**, 1457–1464.
12. Le Marchand, L., Seifried, A., Lum-Jones, A., Donlon, T. and Wilkens, L.R. (2003) Association of the cyclin D1 A870G polymorphism with advanced colorectal cancer. *JAMA*, **290**, 2843–2848.
13. Grieu, F., Malaney, S., Ward, R., Joseph, D. and Iacopetta, B. (2003) Lack of association between CCND1 G870A polymorphism and the risk of breast and colorectal cancers. *Anticancer Res.*, **23**, 4257–4259.
14. Hong, Y., Eu, K.W., Seow, Choen, F., Fook-Chong, S. and Cheah, P.Y. (2005) GC genotype of cyclin D1 G870A polymorphism is associated with increased risk and advanced colorectal cancer in patients in Singapore. *Carcinogenesis*, **41**, 1037–1044.
15. Kong, S., Amos, C.I., Luthra, R., Lynch, P.M., Levin, B. and Frazier, M.L. (2000) Effects of cyclin D1 polymorphism on age of onset of hereditary nonpolyposis colorectal cancer. *Cancer Res.*, **60**, 249–252.
16. Kong, S., Wei, Q., Amos, C.I., Lynch, P.M., Levin, B., Zong, J. and Frazier, M.L. (2001) Cyclin D1 polymorphism and increased risk of colorectal cancer at young age. *J. Natl Cancer Inst.*, **93**, 106–1108.
17. Porter, T.R., Richards, F.M., Houlston, R.S. *et al.* (2002) Contribution of cyclin d1 (CCND1) and E-cadherin (CDH1) polymorphisms to familial and sporadic colorectal cancer. *Oncogene*, **21**, 1928–1933.
18. McKay, J.A., Douglas, J.J., Ross, V.G., Curran, S., Murray, G.I., Cassidy, J. and McLeod, H.L. (2000) Cyclin D1 protein expression and gene polymorphism in colorectal cancer. Aberdeen Colorectal Initiative. *Int. J. Cancer*, **88**, 77–81.
19. Lewis, R.C., Bostick, R.M., Xie, D., Deng, Z., Wargovich, M.J., Fina, M.F., Roufail, W.M. and Geisinger, K.R. (2003) Polymorphism of the cyclin D1 gene, CCND1 and risk for incident sporadic colorectal adenomas. *Cancer Res.*, **63**, 8549–8553.
20. Schernhammer, E.S., Tranah, G.J., Giovanucci, E., Chan, A.T., Ma, J., Colditz, G.A., Hunter, D.J., Willett, W.C. and Fuchs, C.S. (2006) Cyclin D1 A870G polymorphism and the risk of colorectal cancer and adenoma. *Br. J. Cancer*, **94**, 928–934.
21. Seow, A., Yuan, J.-M., Sun, C.-L., Van Den Berg, D., Lee, H.-P. and Yu, M.C. (2002) Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study. *Carcinogenesis*, **12**, 2055–2061.
22. Murillo, G. and Mehta, R.G. (2001) Cruciferous vegetables and cancer prevention. *Nutr. Cancer*, **41**, 17–28.
23. Zhang, Y. and Talalay, P. (1994) Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. *Cancer Res.*, **54**, 1976–1981.
24. Hankin, J.H., Stram, D.O., Arakawa, K., Park, S., Low, S.H., Lee, H.-P. and Yu, M.C. (2001) Singapore Chinese Health Study: development, validation and calibration of the quantitative food frequency questionnaire. *Nutr. Cancer*, **39**, 187–195.
25. Lum, A. and Le Marchand, L. (1998) A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. *Cancer Epidemiol. Biomarker Prev.*, **7**, 719–724.
26. Seow, A., Koh, W.P., Chia, K.S., Shi, L.M., Lee, H.P. and Shanmugaratnam, K.S. (2004) *Trends in Cancer Incidence in Singapore, 1968–2002*. The Singapore Cancer Registry Report No. 6. Singapore.
27. Lee, L.G., Connell, C.R. and Bloch, W. (1993) Allelic discrimination by nick-translation PCR with fluorogenic probes. *Nucleic Acids Res.*, **21**, 3761–3766.
28. Wang, L., Habuchi, T., Takahashi, T. *et al.* (2002) Cyclin D1 gene polymorphism is associated with an increased risk of urinary bladder cancer. *Carcinogenesis*, **23**, 257–264.
29. Breslow, N.E. and Day, N.E. (1980) *Statistical Methods in Cancer Research I: The Analysis of Case-Control Studies*. IARC Scientific Publications, Vol. 32, IARC, Lyon.
30. Hu, X., Xia, H., Srivastava, S.K. *et al.* (1997) Activity of four allelic forms of glutathione S-transferase hGSTP1-1 for diol epoxides of polycyclic hydrocarbons. *Biochem. Biophys. Res. Commun.*, **238**, 397–402.
31. Harries, L.W., Stubbins, M.J., Forman, D., Howard, G.C.W. and Wolf, C.R. (1997) Identification of genetic polymorphisms at the glutathione S-transferase P1 locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis*, **18**, 641–644.
32. Hosmer, W. and Lemeshow, S. (1989) *Polytomous Logistic Regression*. In: Hosmer, W. and Lemeshow, S., (eds) *Applied Logistic Regression*. John Wiley & Sons, New York, pp. 216–245.
33. Shackelford, R.E., Kaufmann, W.K. and Paules, R.S. (2000) Oxidative stress and cell cycle checkpoint function. *Free Radic. Biol. Med.*, **28**, 1387–1404.
34. Burch, P.M. and Heintz, N.H. (2005) Redox regulation of cell-cycle re-entry: cyclin D1 as a primary target for the mitogenic effects of reactive oxygen and nitrogen species. *Antioxid. Redox Signal.*, **7**, 741–751.
35. Loo, G. (2003) Redox-sensitive mechanisms of phytochemical-mediated inhibition of cancer cell proliferation. *J. Nutr. Biochem.*, **14**, 64–73.
36. Bowerman, B. (2005) Oxidative stress and cancer: a β -catenin convergence. *Science*, **308**, 1119–1120.
37. Tetsu, O. and McCormick, F. (1999) β -catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature*, **398**, 422–426.
38. Shen, G., Xu, C., Chen, C., Hebbar, V. and Kong, A.N. (2006) p53-independent G(1) cell cycle arrest of human colon carcinoma cells HT-29 by sulforaphane is associated with induction of p21(CIP1) and inhibition of expression of cyclin D1. *Cancer Chemother. Pharmacol.*, **57**, 317–327.
39. Chiao, J.W., Chung, F.L., Kancherla, R., Ahmed, T., Mittelman, T., Mittelman, A. and Conaway, C.C. (2002) Sulforaphane and its metabolite mediate growth arrest and apoptosis in human prostate cancer cells. *Int. J. Oncol.*, **20**, 631–636.
40. Gamet-Payastre, L., Li, P., Lumeau, S., Cassar, G., Dupont, M.A., Chevrolleau, S., Gasc, N., Tulliez, J. and Terce, F. (2000) Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. *Cancer Res.*, **60**, 1426–1433.
41. Srivastava, S.K. and Singh, S.V. (2004) Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferative activity of benzyl isothiocyanate against human pancreatic cancer cells. *Carcinogenesis*, **25**, 1701–1709.
42. Firestone, G.L. and Bjeldanes, L.F. (2003) Indole-3-carbinol and 3-3'-Diindolylmethane antiproliferative signalling pathways control cell cycle gene transcription in human breast cancer cells by regulating promoter-Sp1 transcription factor interactions. *J. Nutr.*, **133**, 2448S–2455S.
43. Lund, E. (2003) Non-nutritive bioactive constituents of plants: dietary sources and health benefits of glucosinolates. *Int. J. Vitam. Nutr. Res.*, **73**, 135–143.
44. Zhang, Y., Tang, L. and Gonzalez, V. (2003) Selected isothiocyanates rapidly induce growth inhibition of cancer cells. *Mol. Cancer Ther.*, **2**, 1045–1052.
45. Brandi, G., Paiardini, M., Cervasi, B., Fiorucci, C., Filippone, P., De Marco, C., Zaffaroni, N. and Magnani, M. (2003) A new indole-3-carbinol tetrameric derivative inhibits cyclin-dependent kinase 6 expression and induces G1 cell cycle arrest in both estrogen-dependent and estrogen-independent breast cancer cell lines. *Cancer Res.*, **63**, 4028–4036.
46. Wang, L., Liu, D., Ahmed, T., Chung, F.-L., Conaway, C. and Chiao, J.-W. (2004) Targeting cell cycle machinery as a molecular mechanism of sulforaphane in prostate cancer prevention. *Int. J. Oncol.*, **24**, 187–192.
47. Pham, N.-A., Jacobberger, J.W., Schimmer, A.D., Cao, P., Gronda, M. and Hedley, D.W. (2004) The dietary isothiocyanate sulforaphane targets pathways of apoptosis, cell cycle arrest and oxidative stress in human pancreatic cancer cells and inhibits tumor growth in severe combined immunodeficient mice. *Mol. Cancer Ther.*, **3**, 1239–1248.
48. Lin, H.J., Probst-Hensch, N.M., Louie, A.D., Kau, I.H., Witte, J.S., Ingles, S.A., Frankl, H.D., Lee, E.R. and Haile, R.W. (1998) Glutathione transferase null genotype, broccoli and lower prevalence of colorectal adenomas. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 647–652.
49. Pagano, M., Theodoras, A.M., Tam, S.W. and Draetta, G.F. (1994) Cyclin D1-mediated inhibition of repair and replicative DNA synthesis in human fibroblasts. *Genes Dev.*, **8**, 1627–1639.
50. Quelle, D.E., Ashmun, R.A., Shurtleff, S.A., Kato, J.Y., Bar-Sagi, D., Roussel, M.F. and Sherr, C.J. (1993) Overexpression of mouse D-type cyclins accelerates G1-phase in rodent fibroblasts. *Genes Dev.*, **7**, 1559–1571.
51. Kinoshita, A., Wanibuchi, H., Imaoka, S., Ogawa, M., Masuda, C., Morimura, K., Funae, Y. and Fukushima, S. (2002) Formation of 8-hydroxydeoxyguanosine and cell-cycle arrest in the rat liver via generation of oxidative stress by phenobarbital: association with expression profiles of p21(WAF1/Cip1), cyclin D1 and Ogg1. *Carcinogenesis*, **23**, 341–349.
52. Turner, B.C., Gumbs, A.A., Carter, D., Glazer, P.M. and Haffty, B.G. (2000) Cyclin D1 expression and early breast cancer recurrence following lumpectomy and radiation. *Int. J. Radiat. Oncol. Biol. Phys.*, **47**, 1169–1176.

53. Pardo, F.S., Su, M. and Borek, C. (1996) Cyclin D1 induced apoptosis maintains the integrity of the G1/S checkpoint following ionizing radiation irradiation. *Somat. Cell Mol. Genet.*, **22**, 135–144.
54. Coco Martin, J.M., Balkenende, A., Verschoor, T., Lallemand, F. and Michalides, R. (1999) Cyclin D1 overexpression enhances radiation-induced apoptosis and radiosensitivity in a breast tumor cell line. *Cancer Res.*, **59**, 1134–1140.
55. Rowland, B.D. and Peeper, D.S. (2006) KLF4, p21 and context-dependent opposing forces in cancer. *Nat. Rev. Cancer*, **6**, 11–23.
56. Solomon, D.A., Wang, Y., Fox, S.R., Lambeck, T.C. and Giesting, S. (2003) Cyclin D1 splice variants. *J. Biol. Chem.*, **278**, 30339–30347.
57. Sawa, H., Ohshima, T.A., Ukita, H., Murakami, H., Chiba, Y., Kamada, H., Hara, M. and Saito, I. (1998) Alternatively spliced forms of cyclin D1 modulate entry into the cell cycle in an inverse manner. *Oncogene*, **16**, 1701–1712.
58. Kolonel, L.N., Henderson, B.E., Hankin, J.H., Nomura, A.M.Y., Wilkens, L.R., Pike, M.C., Stram, D.O., Monroe, K.R., Earle, M.E. and Nagamine, F.S. (2000) A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. *Am. J. Epidemiol.*, **151**, 346–357.
59. Seow, A., Shi, C.Y., Chung, F.L., Jiao, D., Hankin, J.H., Lee, J.P., Coetzee, G.A. and Yu, M.C. (1998) Urinary isothiocyanate (ITC) in a population-based sample of middle-aged and older Chinese in Singapore: relationship with dietary total ITC and glutathione S-transferase M1/T1/P1 genotypes. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 775–781.
60. Buch, S., Zhu, B., Davis, A.G., Odom, D., Siegfried, J.M., Grandis, J.R. and Romkes, M. (2005) Association of polymorphisms in the cyclin D1 and XPD genes and susceptibility to cancers of the upper aerodigestive tract. *Mol. Carcinog.*, **42**, 222–228.
61. Shu, X.O., Moore, D.B., Cai, Q., Cheng, J., Wen, W., Pierce, L., Cai, H., Gao, Y.T. and Zheng, W. (2005) Association of cyclin D1 genotype with breast cancer risk and survival. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 91–97.

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