Association of *Cyclin D1* Genotypes with Nasopharyngeal Carcinoma Risk

LIANG-CHUN SHIH[1,2*], CHIA-WEN TSAI[2,3*], MING-HSUI TSAI[1,2*], YUNG-AN TSOU[1,2], WEN-SHIN CHANG[2,3], FANG-JING LI[2], MENG-HSUAN LEE[2,4] and DA-TIAN BAU[2,3,4]

[1]Department of Otolaryngology and [2]Terry Fox Cancer Research Laboratory, China Medical University Hospital, Taichung, Taiwan, R.O.C.; [3]Graduate Institute of Basic Medical Science, [4]Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan, R.O.C.

Abstract. *Aim: The cell cycle regulator cyclin D1 (CCND1) is a critical regulator of the G1/S phase transition and plays an important part in several tumor types. This study aimed at investigating the association of CCND1 with and examining the interaction among CCND1 genotype and individual smoking habit in nasopharyngeal carcinoma susceptibility. Patients and Methods: A total of 352 native Taiwanese consisting of 176 cases and 176 controls were enrolled in this hospital-based study, and CCND1 A870G (rs9344) and C1722G (rs678653) genotyping were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and partially verified by direct sequencing. Results: The results showed that there were significant differences between nasopharyngeal carcinoma and control groups in the distribution of the genotypic (p=0.0222) and allelic (p=0.0322) frequencies in the CCND1 A870G genotype. Individuals who carried at least one G allele (GG or AG) had a 0.71-fold lower risk of developing nasopharyngeal carcinoma compared to those who had the AA genotype (95% confidence interval=0.53-0.96). In addition, there is an obvious joint effect of CCND1 A870G genotype with smoking habit on nasopharyngeal carcinoma susceptibility. Conclusion: These findings support the conclusion that the cell cycle regulation may play a role in nasopharyngeal carcinoma development and that CCND1 A870G polymorphism maybe a useful biomarker for nasopharyngeal carcinoma progression.*

*These Authors contributed equally to this study.

Correspondence to: Da-Tian Bau, Ph.D., Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886422053366 Ext 3312, Fax: +886422053366 Ext 1511, e-mail: datian@mail.cmuh.org.tw; artbau2@gmail.com

Key Words: Cyclin D1, polymorphism, nasopharyngeal carcinoma, smoking habit.

Nasopharyngeal carcinoma is the cancer originating in the nasopharynx, the uppermost region of the pharynx, behind the nose where the nasal passages and auditory tubes join the remainder of the upper respiratory tract. Geographically, nasopharyngeal carcinoma occurs sporadically in the West, and more commonly in certain regions of Asia than elsewhere, for instance, Southern China (age standardized rate=30-50/100,000) Southeast Asia (age standardized rate=9-12/100,000) and Taiwan (age standardized rate=8.2-8.4/100,000) (1-3). The geographical pattern of nasopharyngeal carcinoma incidence suggests a unique interaction of Epstein-Barr virus (EBV) viral, dietary and genetic factors (4-9).

Nasopharyngeal carcinoma has been thought to be one of the smoking-related cancers. In literature, smoking has been found to induce oxidative insults to the human genome, with the major DNA adducts of 8-hydroxy-2-deoxyguanine (8-OH-dG) (10, 11). 8-OH-dG is mutagenic, and, if not repaired in time, can cause severe GC to TA transversions in several oncogenes and tumor suppressor genes and, in turn, lead to carcinogenesis (10, 11).

Cyclin D1 (CCND1) plays a critical role in the G1/S phase transition of the cell cycle (12), which accomplishes this critical role by forming a complex with its kinase partners CDK4 or CDK6 (12, 13). Some reports have demonstrated that CCND1 may be involved in the development of some types of tumors in a CDK-independent pattern (14, 15). Dysregulation of CCND1 is a commonly observed characteristic of human cancer, and frequently, overexpression of CCND1 has been reported as a potential biomarker in human cancer, such as oral carcinoma (16-18). However, the underlying mechanisms of CCND1 overexpression and its relationship to nasopharyngeal carcinoma progression are poorly understood. In literature, limited information is available regarding the genetic role of *CCND1* in nasopharyngeal carcinoma, with only some articles on head and neck cancer (19), in oral premalignant lesion (20), and two on oral cancer (21, 22).

Table I. *Characteristics of nasopharyngeal carcinoma patients and controls.*

Characteristic	Controls (n=176)			Patients (n=176)			*p*-value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			49.3 (9.4)			48.2 (11.1)	0.6851
Gender							1.0000
Male	128	72.7%		128	72.7%		
Female	48	27.3%		48	27.3%		
Indulgence							
Cigarette smoking	73	41.5%		77	43.8%		0.7465
Betel quid chewing	54	30.7%		55	31.3%		1.0000
Alcohol drinking	72	40.9%		80	45.5%		0.5414

[a]Based on Chi-square test.

In this study, we aimed at evaluating the contribution of *CCND1* polymorphisms to nasopharyngeal carcinoma in Taiwan. In addition, we also investigated the joint interaction of genotype with smoking behaviors.

Patients and Methods

Study population and sample collection. One hundred and seventy-six cancer patients diagnosed with nasopharyngeal carcinoma were recruited at the outpatient clinics of general surgery between 2003-2009 at the China Medical University Hospital, Taichung, Taiwan. The clinical characteristics of patients including histological details were all graded and defined by expert surgeons. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. As many healthy volunteers as controls were selected, matched for age, gender and habits after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. Both groups completed a short questionnaire which included habits. Smokers were defined as daily or almost daily smokers who had smoked at least five packs of cigarettes in their lifetime. Smokers were recorded for their age of smoking initiation, whether they were currently smoking or had already quit, and if so, when they had quit, and on average, how many cigarettes they smoked or had smoked daily. Our study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants.

Genotyping conditions. Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and stored as previously published (23-29). The primers used for *CCND1* A870G were: forward 5'-GTG AAG TTC ATT TCC AAT CCG C-3', and reverse 5'-GGG ACA TCA CCC TCA CTT AC-3'; for *CCND1* C1722G were: forward 5'-CTC TTG GTT ACA GTA GCG TAG C-3', and reverse 5'-ATC GTA GGA GTG GGA CAG GT-3'. The following cycling conditions were used: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s; and a final extension at 72˚C for 10 min.

Restriction fragment length polymorphism (RFLP) conditions. For *CCND1* A870G, the resultant 167 bp PCR product was mixed with 2 U Nci I and incubated for 3 h at 37˚C. The G form PCR products could be further digested while the A form could not. Two fragments of 145 bp and 22 bp were present if the product was the digestible G form. As for the *CCND1* C1722G, the resultant 159 bp PCR product was mixed with 2 U *Hae* III and incubated for 3 h at 37˚C. On digestion with *Hae* III, the PCR product arising from the G allele was cut into fragments of 111, 26 and 22 bp, whereas C allele was cut into fragments of 137 and 22 bp. Subsequently, 10 μl of product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The genotype analysis was performed by two researchers independently and blindly. Ten percent of the samples were randomly selected for direct sequencing and the results were 100% concordant.

Statistical analyses. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *CCND1* single nucleotide polymorphisms in the control subjects from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's Chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the *CCND1* genotypes between cases and controls. Data was recognized as significant when the statistical *p*-value was less than 0.05.

Results

The characteristics of nasopharyngeal carcinoma patients and the non-cancer controls are summarized in Table I. There were no significant differences between the groups in their age, sex, cigarette smoking, betel quid chewing and alcohol drinking status (Table I). The frequencies of the genotypes and alleles of the CCND1 A870G in the nasopharyngeal carcinoma and control groups are shown in Table II. Firstly, there was a significant difference between nasopharyngeal carcinoma and control groups in the distribution of genotypic frequency (p=0.0222), and the

Table II. *Distribution of CCND1 A870G (rs9344) genetic and allelic frequencies among nasopharyngeal carcinoma patient and control groups. A870G (rs9344).*

	Controls		Patients		OR (95% CI)	*P*-value[a]
	n	%	n	%		
Genetic frequency						
AA	43	24.4%	67	38.1%	1.00 (Reference)	0.0222
AG	105	59.7%	86	48.9%	0.59 (0.36-0.94)	
GG	28	15.9%	23	13.0%	0.56 (0.29-1.10)	
Carrier comparison						
AA+AG	148	84.1%	153	86.9%	1.00 (Reference)	NS
GG	28	15.9%	23	13.1%	0.79 (0.44-1.44)	
AA	43	24.4%	67	38.1%	1.00 (Reference)	0.0080
AG+GG	133	75.6%	109	61.9%	0.42 (0.26-0.68)	
Allelic frequency						
Allele A	191	54.3%	220	62.5%	1.00 (Reference)	0.0322
Allele G	161	45.7%	132	37.5%	0.71 (0.53-0.96)	

OR: Odds ratio, CI: confidence interval; [a]based on Chi-square test, NS: non-significant.

odds ratio of the AG was 0.59 (95% confidence interval, CI=0.36-0.94), compared to the AA wild-type genotype. Secondly, we have performed the dominant and recessive comparison, finding that the odds ratios of the AG+GG *versus* AA were 0.42 (95% CI=0.26-0.68, p=0.0080). Lastly, there was also a significant difference between nasopharyngeal carcinoma and control groups in the distribution of allelic frequency (p=0.0322). To sum up, individuals who carried at least one G allele (AG and GG) had a lower risk of developing nasopharyngeal carcinoma compared to those who carried the AA wild-type genotype (Table II). As for the *CCND1* C1722G, there was no difference in the distributions of either genotype or allelic frequency between nasopharyngeal carcinoma patient and control groups (Table III). The conclusive finding deduced from the data in Tables II and III is that the G allele of *CCND1* A870G seems to be a protective factor for nasopharyngeal carcinoma in Taiwan.

The interaction of the genotype of *CCND1* A870G and cigarette smoking, betel quid chewing, and alcohol drinking habits was of interest. The genotype distribution of various genetic polymorphisms of *CCND1* A870G was significantly different between the nasopharyngeal carcinoma and the control groups who have had a smoking habit (p=0.0165) (Table IV). Consistent with the findings in Table II, the GG genotype frequency was still significantly lower (6.5%) in cancer patients who had smoking habits than in smoking controls (15.0%). There was no such distribution difference in the non-smoking groups (p>0.05). There was no obvious interaction between the genotype of *CCND1* A870G with individual betel quid chewing, and alcohol drinking habits found (data not shown).

Discussion

In order to examine the role of *CCND1* in nasopharyngeal carcinoma, in this study, we selected two common polymorphic sites of the *CCND1* gene, A870G (rs9344) and C1722G (rs678653), and clarified their associations with susceptibility for nasopharyngeal carcinoma risk in central Taiwan. We found that the G variant genotypes of *CCND1* A870G were significantly associated with a lower susceptibility for nasopharyngeal carcinoma (Table II), and this genotype had joint effects with individual smoking habits on nasopharyngeal carcinoma susceptibility (Table IV), while the *CCND1* C1722G genotype may contribute very little to susceptibility to nasopharyngeal carcinoma. At the same time, no obvious joint effect of *CCND1* C1722G genotype with either betel quid chewing or alcohol drinking habits on nasopharyngeal carcinoma risk was found.

Several studies showed that the genotypes of *CCND1* A870G were associated with cancer risk, however, which genotype plays the more critical role remains unclear and it may be disease- and ethnicity-dependent. Consistent with our results in nasopharyngeal carcinoma, the G allele seems to be a protective factor in hepatocellular carcinoma (30), laryngeal (31), breast (32), colorectal (33, 34), and bladder tumors (35). But several controversial findings reported that the G allele was risky in oral (22) and colorectal cancer (36), or not associated in various types of cancer (21, 37-40). There is no denying that the investigated sample sizes of all these studies needed to be enlarged and their finding confirmed by other teams; any conclusion of the genetic role that *CCND1* plays in carcinogenesis can still not easily be made.

Table III. *Distribution of CCND1 C1722G (rs678653) genetic and allelic frequencies among nasopharyngeal carcinoma patient and control groups.*

C1722G (rs678653)	Controls		Patients		OR (95% CI)	*p*-value[a]
	n	%	n	%		
Genetic frequency						
GG	124	70.5%	127	72.2%	1.00 (Reference)	NS
CG	38	21.6%	37	21.0%	0.95 (0.57-1.59)	
CC	14	7.9%	12	6.8%	0.84 (0.37-1.88)	
Carrier comparison						
GG+CG	162	92.1%	164	93.2%	1.00 (Reference)	NS
CC	14	7.9%	12	6.8%	0.85 (0.38-1.89)	
GG	124	70.5%	127	72.2%	1.00 (Reference)	NS
CG+CC	52	29.5%	49	27.8%	0.92 (0.58-1.46)	
Allelic frequency						
Allele G	286	81.3%	291	82.7%	1.00 (Reference)	NS
Allele C	66	18.7%	61	17.3%	0.91 (0.62-1.33)	

OR: Odds ratio, CI: confidence interval; [a]based on Chi-square test, NS: non-significant.

From the proteomic viewpoint, the overexpression of *CCND1* was found to be associated with oral cancer risk (16, 41). However, the underlying mechanism leading to this aberrant expression remains poorly understood. One of the probable mechanisms of CCND1 overexpression is alternate splicing modulated by A870G, which can lead to the sustainment of the CCND1 protein for a longer time (42, 43). Recently, in esophageal adenocarcinomas, the A allele of A870G was found to promote CCND1 expression (44). Contradictory to this study, also performed in head and neck cancer, no association between A870G polymorphism and CCND1 expression was reported in another study (44). The limited sample size of this study leads to some borderline findings (Tables II, III and IV), and should be verified in the near future with an enlarged population. The genotype–phenotype correlation and detailed mechanisms of *CCND1* contributing to nasopharyngeal carcinoma need further investigations.

To sum up, this is so far the earliest study which focuses on *CCND1* and its joint effects with smoking habit on nasopharyngeal carcinoma risk. The genotype of *CCND1* A870G, interacting with smoking habit, may play an important role in the progression of nasopharyngeal carcinoma.

Acknowledgements

We thank Yeong-Ruey Chu, and Tzu-Hsin Weng for their technical assistance. This study was supported by research grants from the China Medical University and Hospital (DMR-101-034), Terry Fox Cancer Research Foundation and the National Science Council (NSC 98-2320-B-039-010-MY3 and 98-2218-E-039-001).

Table IV. *Distribution of CCND1 A870G (rs9344) genotypes in nasopharyngeal carcinoma patients after stratification by cigarette smoking habit.*

Variable	CCND1 A870G (rs9344) genotype			*p*-value[a]
	AA (%)	AG (%)	GG (%)	
Smokers				
Controls	18 (24.7%)	44 (60.3%)	11 (15.0%)	0.0165[b]
Patients	35 (45.5%)	37 (48.0%)	5 (6.5%)	
Non-smokers				
Controls	25 (24.3%)	61 (59.2%)	17 (16.5%)	NS
Patients	32 (31.1%)	49 (47.6%)	18 (17.4%)	

[a]Based on Chi-square test, [b]Statistically significant, NS: non-significant.

References

1 Chan AT, Teo PM and Johnson PJ: Nasopharyngeal carcinoma. Ann Oncol *13*: 1007-1015, 2002.

2 Yu MC and Yuan JM: Epidemiology of nasopharyngeal carcinoma. Semin Cancer Biol *12*: 421-429, 2002.

3 Department of Health, Taiwan: Cancer Registration System Annual Report. Taiwan, Department of Health, 2010.

4 Niedobitek G, Hansmann ML, Herbst H, Young LS, Dienemann D, Hartmann CA, Finn T, Pitteroff S, Welt A and Anagnostopoulos I: Epstein-Barr virus and carcinomas: undifferentiated carcinomas but not squamous cell carcinomas of the nasopharynx are regularly associated with the virus. J Pathol *165*: 17-24, 1991.

5 Zong YS, Sham JS, Ng MH, Ou XT, Guo YQ, Zheng SA, Liang JS and Qiu H: Immunoglobulin A against viral capsid antigen of Epstein-Barr virus and indirect mirror examination of the nasopharynx in the detection of asymptomatic nasopharyngeal carcinoma. Cancer *69*: 3-7, 1992.

6 Mirabelli MC, Hoppin JA, Tolbert PE, Herrick RF, Gnepp DR and Brann EA: Occupational exposure to chlorophenol and the risk of nasal and nasopharyngeal cancers among U.S. men aged 30 to 60. Am J Ind Med *37*: 532-541, 2000.

7 Armstrong RW, Imrey PB, Lye MS, Armstrong MJ, Yu MC and Sani S: Nasopharyngeal carcinoma in Malaysian Chinese: salted fish and other dietary exposures. Int J Cancer *77*: 228-235, 1998.

8 Feng BJ, Huang W, Shugart YY, Lee MK, Zhang F, Xia JC, Wang HY, Huang TB, Jian SW, Huang P, Feng QS, Huang LX, Yu XJ, Li D, Chen LZ, Jia WH, Fang Y, Huang HM, Zhu JL, Liu XM, Zhao Y, Liu WQ, Deng MQ, Hu WH, Wu SX, Mo HY, Hong MF, King MC, Chen Z and Zeng YX: Genome-wide scan for familial nasopharyngeal carcinoma reveals evidence of linkage to chromosome 4. Nat Genet *31*: 395-399, 2002.

9 Feng XL, Zhou W, Li H, Fang WY, Zhou YB, Yao KT and Ren CP: The *DLC-1* -29A/T polymorphism is not associated with nasopharyngeal carcinoma risk in Chinese population. Genet Test *12*: 345-349, 2008.

10 Chen L, Elahi A, Pow-Sang J, Lazarus P and Park J: Association between polymorphism of human oxoguanine glycosylase 1 and risk of prostate cancer. J Urol *170*: 2471-2474, 2003.

11 Xu J, Zheng SL, Turner A, Isaacs SD, Wiley KE, Hawkins GA, Chang BL, Bleecker ER, Walsh PC, Meyers DA and Isaacs WB: Associations between *hOGG1* sequence variants and prostate cancer susceptibility. Cancer Res *62*: 2253-2257, 2002.

12 Sherr CJ: Cancer cell cycles. Science *274*: 1672-1677, 1996.

13 Sherr CJ: D-Type cyclins. Trends Biochem Sci *20*: 187-190, 1995.

14 Coqueret O: Linking cyclins to transcriptional control. Gene *299*: 35-55, 2002.

15 Fu M, Wang C, Li Z, Sakamaki T and Pestell RG: Minireview: Cyclin D1: normal and abnormal functions. Endocrinology *145*: 5439-5447, 2004.

16 Jayasurya R, Sathyan KM, Lakshminarayanan K, Abraham T, Nalinakumari KR, Abraham EK, Nair MK and Kannan S: Phenotypic alterations in Rb pathway have more prognostic influence than p53 pathway proteins in oral carcinoma. Mod Pathol *18*: 1056-1066, 2005.

17 Bova RJ, Quinn DI, Nankervis JS, Cole IE, Sheridan BF, Jensen MJ, Morgan GJ, Hughes CJ and Sutherland RL: Cyclin D1 and $p16^{INK4A}$ expression predict reduced survival in carcinoma of the anterior tongue. Clin Cancer Res *5*: 2810-2819, 1999.

18 Michalides R, van Veelen N, Hart A, Loftus B, Wientjens E and Balm A: Overexpression of cyclin D1 correlates with recurrence in a group of forty-seven operable squamous cell carcinomas of the head and neck. Cancer Res *55*: 975-978, 1995.

19 Holley SL, Parkes G, Matthias C, Bockmuhl U, Jahnke V, Leder K, Strange RC, Fryer AA and Hoban PR: Cyclin D1 polymorphism and expression in patients with squamous cell carcinoma of the head and neck. Am J Pathol *159*: 1917-1924, 2001.

20 Huang M, Spitz MR, Gu J, Lee JJ, Lin J, Lippman SM and Wu X: Cyclin D1 gene polymorphism as a risk factor for oral premalignant lesions. Carcinogenesis *27*: 2034-2037, 2006.

21 Sathyan KM, Nalinakumari KR, Abraham T and Kannan S: Influence of single nucleotide polymorphisms in *H-Ras* and cyclin D1 genes on oral cancer susceptibility. Oral Oncol *42*: 607-613, 2006.

22 Sathyan KM, Nalinakumari KR, Abraham T and Kannan S: *CCND1* polymorphisms (A870G and C1722G) modulate its protein expression and survival in oral carcinoma. Oral Oncol *44*: 689-697, 2008.

23 Chang CH, Chiu CF, Wang HC, Wu HC, Tsai RY, Tsai CW, Wang RF, Wang CH, Tsou YA and Bau DT: Significant association of *ERCC6* single nucleotide polymorphisms with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 5121-5124, 2009.

24 Chang CH, Wang RF, Tsai RY, Wu HC, Wang CH, Tsai CW, Chang CL, Tsou YA, Liu CS and Bau DT: Significant association of *XPD* codon 312 single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 3903-3907, 2009.

25 Liu CJ, Hsia TC, Wang RF, Tsai CW, Chu CC, Hang LW, Wang CH, Lee HZ, Tsai RY and Bau DT: Interaction of cyclooxygenase 2 genotype and smoking habit in Taiwanese lung cancer patients. Anticancer Res *30*: 1195-1199, 2010.

26 Liu CS, Tsai CW, Hsia TC, Wang RF, Liu CJ, Hang LW, Chiang SY, Wang CH, Tsai RY, Lin CC and Bau DT: Interaction of methylenetetrahydrofolate reductase genotype and smoking habit in Taiwanese lung cancer patients. Cancer Genomics Proteomics *6*: 325-329, 2009.

27 Wang HC, Chiu CF, Tsai RY, Kuo YS, Chen HS, Wang RF, Tsai CW, Chang CH, Lin CC and Bau DT: Association of genetic polymorphisms of *EXO1* gene with risk of breast cancer in Taiwan. Anticancer Res *29*: 3897-3901, 2009.

28 Yang MD, Hsu YM, Kuo YS, Chen HS, Chang CL, Wu CN, Chang CH, Liao YM, Wang HC, Wang MF and Bau DT: Significant association of *KU80* single nucleotide polymorphisms with colorectal cancer susceptibility in Central Taiwan. Anticancer Res *29*: 2239-2242, 2009.

29 Chang CH, Chiu CF, Liang SY, Wu HC, Chang CL, Tsai CW, Wang HC, Lee HZ and Bau DT: Significant association of *KU80* single nucleotide polymorphisms with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 1275-1279, 2009.

30 Akkiz H, Bayram S, Bekar A, Akgollu E and Ozdil B: Cyclin D1 G870A polymorphism is associated with an increased risk of hepatocellular carcinoma in the Turkish population: case–control study. Cancer Epidemiol *34*: 298-302, 2010.

31 Rydzanicz M, Golusinski P, Mielcarek-Kuchta D, Golusinski W and Szyfter K: Cyclin D1 gene (*CCND1*) polymorphism and the risk of squamous cell carcinoma of the larynx. Eur Arch Otorhinolaryngol *263*: 43-48, 2006.

32 Canbay E, Eraltan IY, Cercel A, Isbir T, Gazioglu E, Aydogan F, Cacina C, Cengiz A, Ferahman M, Zengin E and Unal H: *CCND1* and *CDKN1B* polymorphisms and risk of breast cancer. Anticancer Res *30*: 3093-3098, 2010.

33 Jiang J, Wang J, Suzuki S, Gajalakshmi V, Kuriki K, Zhao Y, Nakamura S, Akasaka S, Ishikawa H and Tokudome S: Elevated risk of colorectal cancer associated with the AA genotype of the cyclin D1 A870G polymorphism in an Indian population. J Cancer Res Clin Oncol *132*: 193-199, 2006.

34 Grunhage F, Jungck M, Lamberti C, Berg C, Becker U, Schulte-Witte H, Plassmann D, Rahner N, Aretz S, Friedrichs N, Buettner R, Sauerbruch T and Lammert F: Association of familial colorectal cancer with variants in the E-cadherin (*CDH1*) and cyclin D1 (*CCND1*) genes. Int J Colorectal Dis *23*: 147-154, 2008.

35 Yuan L, Gu X, Shao J, Wang M, Zhu Q and Zhang Z: Cyclin D1 G870A polymorphism is associated with risk and clinicopathologic characteristics of bladder cancer. DNA Cell Biol *29*: 611-617, 2010.

36 Yaylim-Eraltan I, Arikan S, Yildiz Y, Cacina C, Ergen HA, Tuna G, Gormus U, Zeybek U and Isbir T: The influence of cyclin D1 A870G polymorphism on colorectal cancer risk and prognosis in a Turkish population. Anticancer Res *30*: 2875-2880, 2010.

37 Gomes CC, Drummond SN, Guimaraes AL, Andrade CI, Mesquita RA and Gomez RS: *P21/WAF1* and cyclin D1 variants and oral squamous cell carcinoma. J Oral Pathol Med *37*: 151-156, 2008.

38 Gangwar R and Mittal RD: Association of selected variants in genes involved in cell cycle and apoptosis with bladder cancer risk in North Indian population. DNA Cell Biol *29*: 349-356, 2010.

39 Jain M, Kumar S, Upadhyay R, Lal P, Tiwari A, Ghoshal UC and Mittal B: Influence of apoptosis (*BCL2, FAS*), cell cycle (*CCND1*) and growth factor (*EGF, EGFR*) genetic polymorphisms on survival outcome: an exploratory study in squamous cell esophageal cancer. Cancer Biol Ther *6*: 1553-1558, 2007.

40 Satinder K, Chander SR, Pushpinder K, Indu G and Veena J: Cyclin D1 (G870A) polymorphism and risk of cervix cancer: a case–control study in north Indian population. Mol Cell Biochem *315*: 151-157, 2008.

41 Jayasurya R, Francis G, Kannan S, Lekshminarayanan K, Nalinakumari KR, Abraham T, Abraham EK and Nair MK: p53, p16 and cyclin D1: molecular determinants of radiotherapy treatment response in oral carcinoma. Int J Cancer *109*: 710-716, 2004.

42 Betticher DC, Thatcher N, Altermatt HJ, Hoban P, Ryder WD and Heighway J: Alternate splicing produces a novel cyclin D1 transcript. Oncogene *11*: 1005-1011, 1995.

43 Sawa H, Ohshima TA, Ukita H, Murakami H, Chiba Y, Kamada H, Hara M and Saito I: Alternatively spliced forms of cyclin D1 modulate entry into the cell cycle in an inverse manner. Oncogene *16*: 1701-1712, 1998.

44 Izzo JG, Wu TT, Wu X, Ensor J, Luthra R, Pan J, Correa A, Swisher SG, Chao CK, Hittelman WN and Ajani JA: Cyclin D1 guanine/adenine 870 polymorphism with altered protein expression is associated with genomic instability and aggressive clinical biology of esophageal adenocarcinoma. J Clin Oncol *25*: 698-707, 2007.

Received December 15, 2011
Revised January 20, 2012
Accepted January 23, 2012