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Identification of variants in cyclin D1 (*CCND1*) and B-Cell CLL/lymphoma 2 (*BCL2*)

Received: 25 March 2004 / Accepted: 15 May 2004 / Published online: 22 July 2004
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Abstract *CCND1* is an important cell-cycle regulatory protein associated with cell proliferation, poor prognosis and recurrence in cancer, while *BCL2* is an important anti-apoptotic protein that plays a vital role in the regulation of the life span by controlling the rate of apoptosis. Recent studies have shown that *CCND1* and *BCL2* may be responsible for the body mass and the regulation of various metabolic processes. In an effort to discover additional polymorphism(s), we scrutinized the genetic polymorphisms in the *CCND1* and *BCL2*. By direct DNA sequencing in 24 individuals, we identified 22 sequence variants within the 16 kb of whole *CCND1* gene: one in exon 4, 17 in introns and four in the 3' UTR region. We also found eight sequence variants within 7.5 kb exon–intron boundaries of *BCL2* gene: one in promoter, three in exon 1, and four in the 3' UTR region. Haplotypes, their frequencies and linkage disequilibrium coefficients ($|D'|$ and r^2), among polymorphisms were estimated. Among identified variants, seven and six variants of *CCND1* and *BCL2* were genotyped in a larger series of subjects ($n=320$). Statistical analyses of *CCND1* and *BCL2* polymorphisms with two metabolic phenotypes revealed no significant association. The information concerning genetic polymorphisms of *CCND1* and *BCL2* might provide valuable information for future genetic studies of diseases.

Keywords *CCND1* · *BCL2* · Polymorphism · Haplotype · Metabolic phenotypes

Introduction

Cyclin D1 (*CCND1*; also named *BCL1* alternatively, MIM 168461), located on chromosome 11q13, plays a crucial role in the transition from G_1 to S phase of the cell cycle as a key cell-cycle regulator (Sherr 1995), while B-cell CLL/lymphoma 2 (*BCL2*, MIM 151430), located on chromosome 18q21.3, protects cells from apoptosis by binding to *BCL2*-associated X (*BAX*) protein (Leiter et al. 2000). *CCND1* is expressed in response to mitogenic signals promoting transition through the restriction point in the G_1 phase of the cell cycle, and its activity reaches a maximum by associating with cyclin-dependent kinase 4 (*CDK4*) and cyclin-dependent kinase 6 (*CDK6*) in mid to late G_1 phase (Sherr 1996). Over-expression of *CCND1*, due to deregulation, leads to propagation and accumulation of cell damage or genetic errors, and consequently contributes to tumor development (Rimokh et al. 1994). Recent studies have shown that the polymorphisms of *CCND1*, especially *870G>A* (*Pro241Pro*), are associated with the risk, early onset and the outcome of multiple malignancies, including colorectal cancer and squamous cell carcinoma of the head and neck (Zheng et al. 2001; Porter et al. 2002). *CCND1 870G>A* polymorphism, located on the splicing region of exon 4, modulates the production of two types of transcripts by affecting *CCND1* splicing (Howe and Lynas 2001). *BCL2* is localized to mitochondria and interferes with programmed cell death independent of promoting cell division (Hockenbery et al. 1990). When *BCL2* is over-expressed, the cell killing induced by various stimuli can be prevented or markedly reduced (Reed 1994). The various anti-apoptotic functions resulting from the different expressions of the *BCL2* protein in lymphocytes seem to be associated with development of autoimmune disease. The

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non-synonymous polymorphism of *BCL2*, *Ala43Thr* ($G > A$), may contribute to resistance to autoimmune disease (Komaki et al. 1998).

Table 1 Clinical profiles of the study subjects

Clinical profiles	Subjects
Number of subjects	320
Age (mean $\pm$ SD)	60 $\pm$ 10
Sex (M/F)	150/170
Body mass index (kg/m ²)	24.3 $\pm$ 3.0
Total cholesterol (mg/dl)	198.7 $\pm$ 36.8

Fig. 1 Gene maps and haplotypes of *CCND1* and *BCL2*. Coding exons are marked by *black blocks* and 5' and 3' UTR by *white blocks*. The first base of translational start site is denoted as nucleotide +1. *Asterisks* indicate polymorphisms genotyped in a larger Korean population ($n = 320$). The frequencies of polymorphisms without larger scale genotyping are based on sequencing data ($n = 24$). *Bold face* indicates polymorphisms that newly identified in this study. Number in parenthesis means distribution of rare allele of polymorphisms and change of amino acid are also indicated. $r^2 = 1$ means absolute LDs among polymorphisms. **a1** Polymorphisms identified in *CCND1* on chromosome 11q13 (Ref. Genome Seq. NT_078088.02). **a2** Haplotypes of *CCND1* and their frequencies among three ethnic groups. Haplotypes with frequencies ≥ 0.003 are presented. Others (1) contain rare haplotypes: CCGDelTCG, CCADelCCG, TCADelTGG, and TAADelCGG. **b1** Polymorphisms identified in *BCL2* on chromosome 18q21.3 (Ref. Genome Seq. NT_025028.13). **b2** Haplotypes of *BCL2* and their frequencies among three ethnic groups

A1. Map of CCND1 gene on chromosome 11q13

B1. Map of BCL2 gene on chromosome 18q21.3

Recent studies have shown that *CCND1* and *BCL2* may influence on the body mass and the regulation of various metabolisms. *CCND1* may affect the body size (Sicinski et al. 1995) and over-expression of *CCND1* in breast cancer cells triggers autonomous growth by controlling cell-cycle exit (Zwijsen et al. 1996). *BCL2* is well known as a mitochondrial membrane protein and might have an unknown role in the regulation of basal mitochondrial energy metabolism (Shimizu et al. 1998; Kowaltowski et al. 2000). Recent evidence suggests that *BCL2* may also affect growth regulation. *BCL2* delays the entry into the cell cycle of mitogen-stimulated B and T lymphocytes, and also delays the serum stimulation-induced entry into S phase of quiescent fibroblasts (O'Reilly et al. 1996). Furthermore, it was presented that the linkage between cholesterol esterification and the control of cell-cycle G1/S transition due to the function of *CCND1* and *BCL2* was plausible, although this relationship is still obscure (Batetta et al. 2003). Although several evidences have shown that metabolic phenotypes may be influenced by those two genes through cell proliferation and apoptosis, direct association of *CCND1* and *BCL2* with metabolic phenotypes has not been investigated.

In this study, we scrutinized the genetic polymorphisms in *CCND1* and *BCL2* by direct sequencing for the sake of the discovery of additional polymorphisms and the evaluation of the relation between the polymorphisms in two genes and metabolic phenotypes.

A2. Haplotypes

Haplotype	+1212 T>C +2893 C>A +6829 G>A (p241P) +7400 insdelG +7576 C>T +10656 G>C +11886 G>A	Freq.		
		Korean	African American	Caucasian
Ht1	T C A del C G G	0.479	0.170	0.380
Ht2	T C G del C G G	0.273	0.010	0.010
Ht3	C C G ins C C G	0.088	0.760	0.260
Ht4	C C G ins C G G	0.067	0.030	0.110
Ht5	C C G del C C G	0.032	0	0
Ht6	T C A del C G A	0.021	0	0
Ht7	T A G del T G G	0.018	0	0
Ht8	T C A ins C C G	0.007	0	0
Ht9	T C G del C C G	0.006	0	0
Ht10	T C A ins C G G	0.003	0	0.030
Ht11	C C G Del C C G	0	0	0.130
Ht12	T C G ins C C G	0	0.020	0.050
Ht13	T C A del C C G	0	0.010	0.030
Others(1)	.	0.004	0	0

B2. Haplotypes

Haplotype	-938 C>A +21 A>G (T7T) +127 G>A (A43T) +300 C>T (A100A) +221280 G>A +223227 G>A	Freq.		
		Korean	African American	Caucasian
Ht1	C A G C G G	0.555	0.792	0.570
Ht2	A A G C G G	0.278	0.115	0.060
Ht3	A G G C G G	0.076	0.042	0.360
Ht4	C A C G G G	0.043	0	0
Ht5	A A G T G G	0.024	0.052	0
Ht6	C A G C A G	0.009	0	0
Ht7	C A G C G A	0.007	0	0
Ht8	A A G C G A	0.004	0	0
Ht9	A A G C A G	0.003	0	0
Ht10	C G G C A G	0	0	0.010

Here, we present 22 and eight genetic variants found in *CCND1* and *BCL2*, respectively. The association analyses with two metabolic phenotypes [body mass index (BMI) and total cholesterol] were also examined with the polymorphisms of *CCND1* and *BCL2* in a Korean population.

Materials and methods

Subjects

A total of 320 unrelated healthy subjects were randomly recruited from an unselected population at Hallym University Sacred Heart Hospital (in routine health check-ups). The study protocol was approved by the Institutional Review Board of the Clinical Research Institute at Hallym University Hospital. Informed consent was obtained from all subjects before drawing blood. All study subjects were examined in the morning after overnight fast. Height and weight were recorded and used for calculation of BMI. Blood samples were drawn for both biochemical measurements of total cholesterol and DNA extraction. The clinical characteristics of the subjects are shown in Table 1.

Sequencing analysis of human *CCND1* and *BCL2* genes

We sequenced the whole *CCND1* gene and exon–intron boundaries of *BCL2* gene, including each promoter region (~1.5 kb), to investigate genetic variants in 24 Korean DNA samples using the ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, Calif., USA). Twenty-nine primer sets of *CCND1* and 16 primer sets of *BCL2* for the amplification and sequencing analysis

were designed based on GenBank sequences (Ref. Genome seq. for *CCND1*; NT_078088.02 released in Jan. 2004, and Ref. Genome seq. for *BCL2*; NT_025028.13 released in January 2004), respectively. Information regarding primers is available on our website (http://www.snp-genetics.com/user/news_content.asp?board_idx=168). Sequence variants were verified by chromatograms. For comparison with other major ethnic groups of allele frequencies of *CCND1* and *BCL2* polymorphisms identified in the Korean population, we also genotyped 50 Caucasian and 50 African–American DNAs obtained from the Human Genetic Cell Repository (<http://locus.umdj.edu/nigms/>).

Genotyping by single-base extension (SBE)

For genotyping of polymorphic sites, amplification and extension primers were designed for SBE (Vreeland et al. 2002). Primer extension reactions were performed with the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems). Information regarding primers is available on our website (http://www.snp-genetics.com/user/news_content.asp?board_idx=168).

Statistics

We examined Lewontin's D' ($|D'|$) and LD coefficient r^2 between all pairs of biallelic loci (Hedrick and Kumar 2001; Hedrick 1987). Haplotypes of each individual were inferred using the algorithm developed by Stephens et al. (2001) (PHASE). Chi-square test was performed for measuring P -values for the difference of distribution of polymorphisms among three major ethnic groups, including Korean, African–American, and Caucasian. Means and standard deviations (SD) of phenotypes, as

Table 2 Single-nucleotide polymorphisms and allele frequencies of *CCND1* and *BCL2* in Korean ($n=320$), African–American ($n=50$) and Caucasian ($n=50$) subjects

Gene	Locus	Amino acid change	HWE ^a	Heterozygosity ^b	Allele frequency			P^*
					Korean	African–American	Caucasian	
CCND1	+1212T>C	Pro241Pro	0.63	0.31	0.19	0.21	0.50	<0.001
	+2893C>A		0.93	0.04	0.02	0.00	0.00	NS
	+6829G>A		1.00	0.50	0.49	0.18	0.44	<0.001
	+7400 insdelG		0.59	0.28	0.17	0.19	0.45	<0.001
	+7576C>T		0.05	0.04	0.02	0.00	0.00	NS
	+10656G>C		0.92	0.24	0.14	0.21	0.34	<0.001
BCL2	+11886G>A	Thr7Thr Ala43Thr Ala100Ala	0.93	0.04	0.02	0.00	0.00	NS
	–938C>A		0.41	0.48	0.40	0.21	0.42	0.001
	+21A>G		1.00	0.14	0.08	0.04	0.37	<0.001
	+127G>A		0.96	0.10	0.05	0.00	0.00	0.003
	+300C>T		0.19	0.05	0.03	0.05	0.00	NS
	+221280G>A		0.97	0.02	0.01	0.00	0.01	NS
	+223227G>A		0.97	0.02	0.01	0.00	0.00	NS

* P -values of deviation from Hardy–Weinberg equilibrium in the Korean population. Additional information regarding primers is available at http://www.snp-genetics.com/user/news_content.asp?board_idx=168

^aHeterozygosity calculated in Korean population

^b P -value for the difference of distribution of polymorphisms among three major ethnic groups, NS not significant ($P>0.05$)

Table 3 Regression analysis for age-adjusted and sex-adjusted phenotype with *CCND1* and *BCL2* polymorphisms in the Korean population ($n=320$). Genotype and haplotype distributions, means $\pm$ SD of each value, and P -values of codominant models for regression analyses controlling for age (continuous value) and sex (male = 0 and female = 1) as covariates are shown

Gene	Loci	BMI (kg/m ²)		Total cholesterol (mg/dl)					
		C/C ^a	C/R	R/R	P	C/C	C/R	R/R	P
CCND1	+1212T>C	209(24.3 ± 3.1)	92(24.3 ± 2.9)	14(24.9 ± 1.9)	0.50	209(198.6 ± 38.4)	92(195.5 ± 32.6)	14(219.2 ± 39.1)	0.47
	+2893C>A	303(24.3 ± 3.0)	13(24.4 ± 3.5)		0.52	303(198.2 ± 37.0)	13(206.5 ± 37.2)		0.37
	+6829G>A	83(24.3 ± 3.0)	158(24.4 ± 3.1)	75(24.1 ± 2.6)	0.93	84(197.5 ± 36.3)	157(198.8 ± 37.3)	75(199.4 ± 37.6)	0.74
	(Pro241Pro)								
	+7400 insdelG	220(24.3 ± 3.1)	80(24.4 ± 3.0)	11(24.1 ± 2.1)	0.98	219(198.8 ± 38.6)	81(195.9 ± 33.6)	11(212.3 ± 35.4)	0.89
	+7576C>T	302(24.3 ± 3.0)	11(25.2 ± 4.1)	1(23.0 ± 0.0)	0.22	302(198.2 ± 37.1)	11(210.1 ± 34.4)	1(232.0 ± 0.0)	0.15
	+10787G>C	234(24.3 ± 3.1)	75(24.4 ± 2.8)	5(24.7 ± 2.0)	0.63	233(198.9 ± 37.6)	76(197.2 ± 35.5)	5(218.4 ± 31.5)	0.87
	+11886G>A	303(24.3 ± 3.0)	13(25.1 ± 3.0)		0.27	303(198.6 ± 37.0)	13(198.1 ± 36.9)		0.91
	ht1	81(24.1 ± 2.6)	159(24.4 ± 3.2)	71(24.3 ± 2.9)	0.89	81(198.3 ± 37.5)	159(198.9 ± 37.5)	71(198.7 ± 36.4)	0.91
	ht2	161(24.5 ± 3.0)	130(24.2 ± 3.1)	20(23.8 ± 2.9)	0.39	162(200.4 ± 35.3)	129(196.7 ± 39.5)	20(198.4 ± 37.7)	0.53
BCL2	-938C>A	108(24.3 ± 3.1)	164(24.5 ± 3.0)	45(23.9 ± 2.8)	0.79	109(203.2 ± 41.0)	163(196.9 ± 34.0)	45(193.8 ± 36.4)	0.13
	+21A>G	267(24.2 ± 2.9)	45(25.4 ± 3.7)	2(23.7 ± 0.2)	0.03	267(198.6 ± 36.0)	45(195.9 ± 40.5)	2(274.5 ± 20.5)	0.55
	(Thr7Thr)								
	+127G>A	250(24.3 ± 3.1)	26(24.8 ± 2.9)	1(25.8 ± 0.0)	0.26	249(197.7 ± 36.9)	27(201.0 ± 26.9)	1(158.0 ± 0.0)	0.93
	(Ala43Thr)								
	+300C>T	302(24.3 ± 3.0)	14(24.4 ± 3.9)	1(24.7 ± 0.0)	0.82	302(199.7 ± 36.8)	14(175.9 ± 35.1)	1(212.0 ± 0.0)	0.06
	(Ala100Ala)								
	+221280G>A	309(24.3 ± 3.0)	8(24.6 ± 3.0)		0.70	309(198.6 ± 37.2)	8(200.3 ± 25.0)		0.88
	+223227G>A	309(24.3 ± 3.0)	7(26.0 ± 1.7)		0.15	308(198.6 ± 36.6)	8(200.8 ± 53.0)		0.92
	ht1	62(24.2 ± 2.7)	166(24.5 ± 3.1)	88(24.1 ± 3.0)	0.48	63(197.5 ± 36.0)	165(195.9 ± 33.0)	88(204.5 ± 43.9)	0.22
ht2	155(24.7 ± 3.2)	140(24.0 ± 2.9)	21(23.9 ± 1.8)	0.10	156(200.9 ± 42.1)	139(197.1 ± 31.5)	21(192 ± 29.7)	0.29	

^aC/C, C/R and R/R represent homozygotes for common allele, heterozygotes and homozygotes for rare allele, respectively. Haplotypes were inferred using the algorithm developed by Stephens et al. (2001). Missing genotype data were omitted for exact haplotype construction

well as *P*-values were calculated by multiple regression analyses controlling age (continuous value) and sex (male=0 and female=1) as covariates.

Results and discussion

CCND1 (BCL1), a cell-cycle regulatory protein, is associated with cell proliferation, prognosis and recurrence of cancer and its over-expression is often found in many different types of tumors (Coco Martin et al. 1999). BCL2 is an anti-apoptotic protein and plays an important role in the regulation of the rate of apoptosis (Sugino et al. 2000). Despite of the important features of these two genes, only few polymorphisms, including 870G>A and 1722G>C in *CCND1* (Betticher et al. 1995; Holley et al. 2001) and *Ala43Thr* (G>A) in *BCL2* (Komaki et al. 1998), had been reported.

We identified 22 sequence variants within the 16 kb of whole *CCND1* including the -1500 bp promoter region: one in intron 1, two in intron 2, seven in intron 3, one in exon 4, seven in intron 4, and four in the 3' UTR region. We also found eight sequence variants within 7.5 kb exons and their boundaries of *BCL2*, including the -1500 bp promoter region: one in promoter, three in exon 1, and four in the 3' UTR region (Fig. 1 a1,b1). *CCND1* +6829G>A (*Pro241Pro*), *CCND1* +10656G>C, and *BCL2* +127G>A (*Ala43Thr*), in this study, correspond to *CCND1* 870G>A, *CCND1* 1722G>C, and *BCL2* *Ala43Thr* (G>A) in previous reports (Betticher et al. 1995; Komaki et al. 1998; Holley et al. 2001), respectively.

Seven polymorphisms in *CCND1* and six in *BCL2* were selected for a larger scale genotyping (*n*=320) by considering their allele frequencies, haplotype-tagging status, and LDs among polymorphisms. The frequencies of those 13 polymorphisms of *CCND1* and *BCL2* genes were shown in Table 2. The significant differences in frequencies of polymorphisms and haplotypes were observed among three ethnic groups (*P*=0.03 to <0.001) (Table 2; Fig. 1 a2,b2). Linkage disequilibrium coefficients ($|D'|$) and r^2 , among polymorphisms were also calculated (data not shown; information about LDs among polymorphisms is available at http://www.snp-genetics.com/user/news_content.asp?board_idx=168).

Positive associations of *CCND1* 870G>A polymorphism [+6829G>A (*Pro241Pro*), in this study], located on the splice donor region of exon 4, with a wide variety of cancers have been suggested in different populations including Chinese (Deng et al. 2002), Japanese (Wang et al. 2003), and Caucasian (Zheng et al. 2001; Porter et al. 2002). It has been also reported that *CCND1* 1722G>C (+10656G>C in this study) is associated with both poorly differentiated tumors and reduced disease-free interval independent of the influence of *CCND1* 870G>A (Holley et al. 2001). A positive association of *BCL2* *Ala43Thr* (G>A) polymorphism [+127G>A (*Ala43Thr*) in this study] with autoimmune diseases such as type 1 diabetes mellitus (T1DM) has

been reported in the Japanese population (Komaki et al. 1998), whereas there was no association with Caucasian (Heding et al. 2001).

The associations of *CCND1* and *BCL2* polymorphisms (seven polymorphisms and two major haplotypes in *CCND1*, and six polymorphisms and two major haplotypes in *BCL2*) with metabolic phenotypes (BMI and total cholesterol) were analyzed in this study. The results of statistical analyses are summarized in Table 3. In association analysis of cholesterol with *CCND1* and *BCL2* polymorphisms based on the evidence that cholesterol was related to the cell replication and apoptotic cell death mainly due to the functional distribution of *CCND1* and *BCL2* genes (Kockx et al. 1998), we could not find any association. On the other hand, among polymorphisms analyzed, *BCL2* +21A>G (*Thr7Thr*) showed a weak association with BMI (*P*=0.03; Table 3). The highest BMI was found in +21AG genotypes, intermediate in +21AA genotypes, and the lowest in +21GG genotypes. However, this association of *BCL2* +21A>G with BMI might be controversial because the *P*-value did not reach the significance level when multiple comparison tests were applied. Although it had been proposed that the modulation of the cell cycle and apoptosis of *CCND1* and *BCL2* was associated with increased BMI (Suga et al. 2001), polymorphisms of *CCND1* and *BCL2* including *BCL2* +21A>G, seem to be not responsible for the association with cell cycle and apoptosis through change in levels of BMI. Additional studies would be needed to clarify the association with BMI.

In summary, we identified 22 and eight polymorphic sites in the human *CCND1* and *BCL2* genes, respectively. Among variants of two genes, seven variants in *CCND1* and six in *BCL2* were genotyped in larger scale subjects (*n*=320). Haplotypes, their frequencies and linkage disequilibrium coefficients ($|D'|$ and r^2), were estimated. Using statistical analyses to evaluate the associations of *CCND1* and *BCL2* polymorphisms with two metabolic phenotypes, no reliable associations were found. This information about genetic polymorphisms in *CCND1* and *BCL2* might be useful for host genetic studies of other diseases, especially cancer and apoptosis.

Acknowledgements This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ10-PG6-AG01-0003).

References

- Batetta B, Mulas MF, Sanna F, Putzolu M, Bonatesta RR, Gasperi-Campani A, Roncuzzi L, Baiocchi D, Dessi S (2003) Role of cholesterol ester pathway in the control of cell cycle in human aortic smooth muscle cells. *FASEB J* 17:746-748
- Betticher DC, Thatcher N, Altermatt HJ, Hoban P, Ryder WD, Heighway J (1995) Alternate splicing produces a novel cyclin D1 transcript. *Oncogene* 11:1005-1011
- Coco Martin JM, Balkenende A, Verschoor T, Lallemand F, Michalides R (1999) Cyclin D1 overexpression enhances

- radiation-induced apoptosis and radiosensitivity in a breast tumor cell line. *Cancer Res* 59:1134–1140
- Deng L, Zhao XR, Pan KF, Wang Y, Deng XY, Lu YY, Cao Y (2002) Cyclin D1 polymorphism and the susceptibility to NPC using DHPLC. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)* 34:16–20
- Heding PE, Karlsen AE, Veijola R, Nerup J, Pociot F (2001) No evidence of a functionally significant polymorphism of the BCL2 gene in Danish, Finnish and Basque type 1 diabetes families. *Genes Immun* 2:398–400
- Hedrick PW (1987) Gametic disequilibrium measures: proceed with caution. *Genetics* 117:331–341
- Hedrick P, Kumar S (2001) Mutation and linkage disequilibrium in human mtDNA. *Eur J Hum Genet* 9:969–972
- Hockenbery D, Nunez G, Millman C, Schreiber RD, Korsmeyer SJ (1990) Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* 348:334–336
- Holley SL, Parkes G, Matthias C, Bockmuhl U, Jahnke V, Leder K, Strange RC, Fryer AA, Hoban PR (2001) Cyclin D1 polymorphism and expression in patients with squamous cell carcinoma of the head and neck. *Am J Pathol* 159:1917–1924
- Howe D, 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
- Kockx MM, De Meyer GR, Buysens N, Knaapen MW, Bult H, Herman AG (1998) Cell composition, replication, and apoptosis in atherosclerotic plaques after 6 months of cholesterol withdrawal. *Circ Res* 83:378–387
- Komaki S, Kohno M, Matsuura N, Shimadzu M, Adachi N, Hoshida R, Nishiyama S, Matsuda I (1998) The polymorphic 43Thr bcl-2 protein confers relative resistance to autoimmunity: an analytical evaluation. *Hum Genet* 103:435–440
- Kowaltowski AJ, Vercesi AE, Fiskum G (2000) Bcl-2 prevents mitochondrial permeability transition and cytochrome c release via maintenance of reduced pyridine nucleotides. *Cell Death Differ* 7:903–910
- Leiter U, Schmid RM, Kaskel P, Peter RU, Krahn G (2000) Antiapoptotic bcl-2 and bcl-xL in advanced malignant melanoma. *Arch Dermatol Res* 292:225–232
- O'Reilly LA, Huang DC, Strasser A (1996) The cell death inhibitor Bcl-2 and its homologues influence control of cell cycle entry. *EMBO J* 15:6979–6990
- Porter TR, Richards FM, Houlston RS, Evans DG, Jankowski JA, Macdonald F, Norbury G, Payne SJ, Fisher SA, Tomlinson I, Maher ER (2002) Contribution of cyclin d1 (CCND1) and E-cadherin (CDH1) polymorphisms to familial and sporadic colorectal cancer. *Oncogene* 21:1928–1933
- Reed JC (1994) Bcl-2 and the regulation of programmed cell death. *J Cell Biol* 124:1–6
- Rimokh R, Berger F, Bastard C, Klein B, French M, Archimbaud E, Rouault JP, Santa Lucia B, Duret L, Vuillaume M et al (1994) Rearrangement of CCND1 (BCL1/PRAD1) 3' untranslated region in mantle-cell lymphomas and t(11q13)-associated leukemias. *Blood* 83:3689–3696
- Sherr CJ (1995) D-type cyclins. *Trends Biochem Sci* 20:187–190
- Sherr CJ (1996) Cancer cell cycles. *Science* 274:1672–1677
- Shimizu S, Eguchi Y, Kamiike W, Funahashi Y, Mignon A, Lacronique V, Matsuda H, Tsujimoto Y (1998) Bcl-2 prevents apoptotic mitochondrial dysfunction by regulating proton flux. *Proc Natl Acad Sci USA* 95:1455–1459
- Scinski P, Donaher JL, Parker SB, Li T, Fazeli A, Gardner H, Haslam SZ, Bronson RT, Elledge SJ, Weinberg RA (1995) Cyclin D1 provides a link between development and oncogenesis in the retina and breast. *Cell* 82:621–630
- Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 68:978–989
- Suga K, Imai K, Eguchi H, Hayashi S, Higashi Y, Nakachi K (2001) Molecular significance of excess body weight in postmenopausal breast cancer patients, in relation to expression of insulin-like growth factor I receptor and insulin-like growth factor II genes. *Jpn J Cancer Res* 92:127–134
- Sugino N, Suzuki T, Kashida S, Karube A, Takiguchi S, Kato H (2000) Expression of Bcl-2 and Bax in the human corpus luteum during the menstrual cycle and in early pregnancy: regulation by human chorionic gonadotropin. *J Clin Endocrinol Metab* 85:4379–4386
- Vreeland WN, Meagher RJ, Barron AE (2002) Multiplexed, high-throughput genotyping by single-base extension and end-labeled free-solution electrophoresis. *Anal Chem* 74:4328–4333
- Wang L, Habuchi T, Mitsumori K, Li Z, Kamoto T, Kinoshita H, Tsuchiya N, Sato K, Ohya C, Nakamura A, Ogawa O, Kato T (2003) Increased risk of prostate cancer associated with AA genotype of cyclin D1 gene A870G polymorphism. *Int J Cancer* 103:116–120
- Zheng Y, Shen H, Sturgis EM, Wang LE, Eicher SA, Strom SS, Frazier ML, Spitz MR, Wei Q (2001) Cyclin D1 polymorphism and risk for squamous cell carcinoma of the head and neck: a case-control study. *Carcinogenesis* 22:1195–1199
- Zwijsen RM, Klompaker R, Wientjens EB, Kristel PM, van der Burg B, Michalides RJ (1996) Cyclin D1 triggers autonomous growth of breast cancer cells by governing cell cycle exit. *Mol Cell Biol* 16:2554–2560