Hematology, 2005; 10 Supplement 1: 117–126

Taylor & Francis
Taylor & Francis

MULTIPLE MYELOMA

Amplification and overexpression of *CKS1B* at chromosome band 1q21 is associated with reduced levels of $p27^{Kip1}$ and an aggressive clinical course in multiple myeloma

JOHN SHAUGHNESSY

Abstract
The molecular basis for aggressive transformation of multiple myeloma (MM) and other cancers is not completely understood. Global gene expression profiling on highly purified malignant plasma cells from 351 newly diagnosed patients with MM treated with autologous stem cell transplantation revealed a statistically significant over-representation of chromosome 1 genes in a group of 70 genes whose expression was linked to poor outcome. In particular, over-expression of *CKS1B*, which maps to an amplicon at 1q21 in myeloma and regulates SCF^{Skp2}-mediated ubquitination and proteolysis of the cyclin dependent kinase inhibitor $p27^{Kip1}$ was significantly over-expressed in patients with poor survival. Interphase fluorescence in-situ hybridization revealed that *CKS1B* expression was strongly correlated with DNA copy number in a subset of 197 cases ($P < 0.0001$) with both measurements. Validated in 224 patients lacking expression analysis, *CKS1B* gene amplification conferred a poor prognosis ($P < 0.0001$) and was an independent predictor of outcome in multivariate analyses ($P = 0.002$). *CKS1B* mRNA and protein expression were correlated and both were inversely correlated with $p27^{Kip1}$ protein levels. RNA interference of *CKS1B* messenger RNA in myeloma cell lines led to reduced *CKS1B* mRNA and protein, an accumulation of $p27^{Kip1}$, and profound growth inhibition. Based on these data we conclude that over-expression of *CKS1B*, mainly due to gene amplification, imparts a poor prognosis in MM, possibly as a result of enhanced degradation of $p27^{Kip1}$

Keywords: *multiple myeloma, prognosis, gene expression profiling, DNA amplification, chromosome 1q21,* CKS1B, *$p27^{Kip1}$, cell cycle regulation.*

Multiple Myeloma is a malignancy of antibody-secreting plasma cells that expand in the bone marrow causing severe osteolytic bone disease and a constellation of additional complications including hypocalcaemia, immunosuppression, anemia, and kidney failure [1]. Although outcome has been greatly improved through the use of high dose melphalan and autologous stem cell transplantations, survival is still highly variable with some patients surviving months and others greater than 10 years or more [2–4]. Aggressive disease, with increased proliferation and a higher frequency of abnormal metaphase karyotypes, elevated LDH and extra-medullary manifestations, seen in approximately 20% of newly diagnosed patients, inevitably appears in all cases, however at a variable rate of onset [4].

Recurrent, non-random genetic lesions have been identified in myeloma and these have been related to clinical course and response to therapy [5]. At the genetic level myelomas can be broadly separated into hyperdiploid and non-hyperdiploid diseases [6]. Non-hyperdiploid myelomas, typically harboring immunoglobulin-mediated translocations leading to transcriptionally activation of *CCND1, CCND3, MAF, MAFB,* or *FGFR3*/*MMSET* are seen in approximately 40% of cases [7–10]. The remaining 60% of myelomas are hyperdiploid with anueploidy resulting from trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19 and 21 [11–14]. Gene expression studies have revealed that virtually all myelomas, regardless of ploidy status, exhibit deregulated expression of one of the three cyclin D genes, suggesting that cyclin D activation may be an initiating genetic event in this malignancy [12]. Myelomas with translocations resulting in activation of *CCND1* are typically diploid and have a more favorable prognosis than those with translocations

Correspondence: John D. Shaughnessy, Donna D, and Donald M, Lambert Laboratory of Myeloma Genetics, Room 915, Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock, AR, 72205. Tel: 501-296-1503, X1457; Fax: 501-686-6442; E-mail: shaughnessyjohn@uams.edu

ISSN 1024-5332 print/ISSN 1607-8454 online
DOI: 10.1080/10245330512331390140

activating *MAF* or *FGFR3/MMSET* [15–18]. Deletion of chromosome 13q14, which is strongly linked to *IGH*-mediated translocations, chromosome 17p and hypodiploidy are associated with a poor prognosis [18–22]. Hyperdiploid tumors are thought to be more dependent on interactions with the bone marrow microenvironment as evidenced by higher levels of DKK1 expression, increased incidence of lytic bone lesions and their conspicuous absence in myeloma cell lines [12,23,24].

The fact that virtually all of the recurrent genetic lesions seen in myeloma are also observed in the benign plasma cell dyscrasia monoclonal gammopathy of undetermined significance (MGUS) [26–28] and that the global gene expression profiles of the two disease are indistinguishable [35], suggests that additional, as yet uncharacterized, gene mutations may be required for progression. Tandem duplications and jumping translocations of the q21 band of chromosome 1 are acquired during myeloma disease progression [29,30] and 1q amplifications have been linked to a poor prognosis in this disease [18,21,31]. No other chromosome or chromosome band undergoes the types of extensive and continual rearrangements as seen for 1q21. Deletion of 17p and acquisition of *MYC* activating translocations are also seen in end stage disease, however a definitive role of these lesions in progression has not been determined.

Global RNA expression profiling is capable of identifying DNA copy number changes in myeloma [12,21] and other cancers [31–33], and unlike array-based comparative genomic hybridization (aCGH) has the added value of pointing to genes whose expression may be altered by these changes. Furthermore, unlike aCGH, RNA profiling can identify gene-activating translocations, not resulting in gain or loss of DNA, that are frequently observed in lymphoid malignancies.

In an effort to identify genes linked to an aggressive clinical course, we applied RNA from highly purified plasma cells derived from 351 newly diagnosed patients with multiple myeloma to Affymetrix U133Plus 2.0 microarrays. Expression extremes of ~54,000 probe sets were correlated with disease-related and overall survival following 2 cycles of high-dose melphalan and autologous stem cell transplantation. Using log rank tests, 70 probe sets were identified for which expression in the fourth or first quartile was correlated with a high incidence of disease-related death. Although 10% of the genes on the microarray were derived from chromosome 1, 30% of the retained genes were derived from this chromosome ($P < 0.0001$). As rearrangements of chromosome 1 have been linked to poor prognosis in myeloma and tandem duplications and jumping translocations of the q21 band of chromosome 1 increase during myeloma progression, we focused on genes from the 1q21 in the list of 70 as candidate genes whose increased expression may be related to increased DNA copy number and leading to disease progression. Two genes, *PSMD4* and *CKS1B*, map to the 1q21 band, and of these two, *CKS1B* was most strongly associated with survival in unadjusted log rank tests (i.e. according to the list order of Table I). There were 40 deaths among 88 patients with *CKS1B* expression in quartile 4 compared to 78 among 263 patients with quartile 1–3 expression ($P < 0.0001$, false discovery rate, 2.5%). To determine if elevated expression was linked to DNA amplification we performed interphase fluorescence *in situ* hybridization analysis on 197 of the 351 cases studied by microarray. *CKS1B* amplification was evident in 46% with the percentage of cases with amplification increasing in frequency as *CKS1B* expression levels increased from quartile 1 to quartile 4 ($P < 0.0001$). These data also showed that *CKS1B* expression significantly increased as CKS1B copy numbers increased from 2 to 3 to greater than equal to 4 copies. We next investigated whether *CKS1B* amplification was associated with survival and event-free survival in a cohort of 224 patients enrolled on the same protocol prior to the initiation of gene expression profiling. *CKS1B* amplification levels were inversely correlated with both event-free survival ($P < 0.0001$, data not shown) and overall survival in this cohort ($P < 0.0001$). These effects were also observed when we combined all 421 (197+224) patients (event-free survival, $P < 0.0001$; data not shown; overall survival, $P < 0.0001$). To test whether *CKS1B* amplification was simply mirroring amplification of the entire chromosome we correlated interphase FISH data with metaphase cytogenetics. As expected, 16 of 17 (94%) cases with evidence of 1q gain by metaphase cytogenetics exhibited amplification by FISH. Importantly, *CKS1B* amplification was also observed by interphase FISH in 61 of 112 cases (54%) lacking evidence of chromosome 1q gain by metaphase cytogenetics ($P < 0.0001$). Given that the incidence of chromosome 1q21 amplification and jumping translocations increases during disease progression and all myeloma cell lines are derived from end stage disease, we evaluated *CKS1B* amplification in a panel of 22 myeloma cell lines. FISH analysis revealed 3 to 8 copies of *CKS1B* in 21 of 22 myeloma cell lines (data not shown). We next investigated whether *CKS1B* expression increased during disease progression. *CKS1B* expression at diagnosis and at relapse, evaluated in 44 paired cases, revealed increased expression in 80% ($P = 0.0001$) with the most dramatic increases occurring in those cases with Q1–Q3 expression at diagnosis. CKS1B FISH on paired baseline and relapse samples from 30 patients enrolled on the protocol showed that of twelve cases lacking amplification at diagnosis, 6 had $>=3$ copies at relapse; of 12 cases with three copies at diagnosis, six had $>=4$ copies at relapse; and of six cases with

Table Ia. Quartile 4 FDR 2.5% gene probe sets – rank correlations with 1q21 amplification index, CKS1B and PC labeling index and adjusted *P*-values for associations with overall survival

Rank (Q4)	Chromosome	Probe set	Symbol	CKS1B amplification index r[†]	CKS1B r[‡]	PCLIr[⋆]	Adjusted survival *P*-value[a]
1	8q21.13	202345_s_at	NA	0.20	0.22		0.001
2	**Xp22.2-p22.1**	**1555864_s_at**	***NA***	**0.34**	**0.47**		**0.007**
3	**5p15.33**	**204033_at**	***TRIP13***	**0.19**	**0.45**	**0.20**	**0.001**
4	1q22	206513_at	*AIM2*	0.15	0.13		0.089
5	2p24.1	1555274_a_at	*SELI*	0.28	0.31		0.001
6	21q22.3	211576_s_at	*SLC19A1*	0.17	0.23		0.007
7	3p21.3	204016_at	*LARS2*	−0.18			0.002
8	1q43	1565951_s_at	*OPN3*	0.36	0.36		0.007
9	**1q31**	**219918_s_at**	***ASPM***	**0.36**	**0.64**	**0.17**	**0.010**
10	**12q15**	**201947_s_at**	***CCT2***	**0.23**	**0.43**	**0.13**	**0.004**
11	16p13.3	213535_s_at	*UBE2I*		0.38		0.022
12	**20q13.2-q13.3**	**204092_s_at**	***STK6***	**0.31**	**0.51**	**0.19**	**0.044**
13	1p36.33-p36.21	213607_x_at	*FLJ13052*				0.150
14	Xq12-q13	208117_s_at	*FLJ12525*		0.34		0.006
15	17q25	210334_x_at	*BIRC5*	0.20	0.36	0.14	0.110
16	**3q27**	**204023_at**	***NA***	**0.29**	**0.62**	**0.16**	**0.072**
17	**1q21.2**	**201897_s_at**	***CKS1B***	**0.50**	**1.00**	**0.15**	**0.007**
18	19q13.11-q13.12	216194_s_at	*CKAP1*	0.24	0.38		0.001
19	**1q21**	**225834_at**	***MGC57827***	**0.39**	**0.66**	**0.23**	**0.140**
20	19q13.12	238952_x_at	*DKFZp779O175*		0.11		0.009
21	**17p13.3**	**200634_at**	***PFN1***	**0.30**	**0.41**		**0.002**
22	19p13.2	208931_s_at	*ILF3*	0.22	0.22		0.220
23	1q22	206332_s_at	*IFI16*	0.30	0.32	0.13	0.003
24	7p14-p13	220789_s_at	*TBRG4*		0.13	0.17	0.009
25	10p11.23	218947_s_at	*PAPD1*	0.31	0.30		0.150
26	8q24	213310_at	*EIF2C2*	0.28	0.37		0.031
27	3q12.1	224523_s_at	*MGC4308*	0.17	0.24	0.14	0.038
28	1p36.3-p36.2	201231_s_at	*ENO1*		0.23		< 0.001
29	18q12.1	217901_at	*DSG2*	0.15			0.005
30	**6q22**	**226936_at**	***NA***	**0.15**	**0.52**	**0.17**	**0.027**
31	8q24.3	58696_at	*EXOSC4*		0.20		0.330
32	**1q21-q25**	**200916_at**	***TAGLN2***	**0.47**	**0.52**		**0.120**
33	3q21	201614_s_at	*RUVBL1*	0.16	0.14		0.023
34	16q22-q24	200966_x_at	*ALDOA*	0.21	0.28		0.001
35	2p25.1	225082_at	*CPSF3*		0.39		0.073
36	1q43	242488_at	*NA*	0.18	0.27	0.14	0.090
37	3q12.3	243011_at	*MGC15606*		0.27		0.004
38	22q13.1	201105_at	*LGALS1*		0.31		0.051
39	**3p25-p24**	**224200_s_at**	***RAD18***	**0.17**	**0.41**	**0.14**	**0.040**
40	20p11	222417_s_at	*SNX5*				0.085
41	**1q21.2**	**210460_s_at**	***PSMD4***	**0.58**	**0.59**	**0.13**	**0.067**
42	**12q24.3**	**200750_s_at**	***RAN***	**0.22**	**0.40**		**0.056**
43	**1pter-q31.3**	**206364_at**	***KIF14***	**0.41**	**0.57**	**0.25**	**0.019**
44	7p15.2	201091_s_at	*CBX3*	0.14	0.20	0.16	0.150
45	**12q22**	**203432_at**	***TMPO***	**0.32**	**0.59**	**0.18**	**0.007**
46	**17q24.2**	**221970_s_at**	***DKFZP586L0724***	**0.27**	**0.47**		**0.081**
47	**11p15.3-p15.1**	**212533_at**	***WEE1***	**0.20**	**0.54**	**0.13**	**0.056**
48	3p12	213194_at	*ROBO1*				0.150
49	5q32-q33.1	244686_at	*TCOF1*				0.120
50	8q23.1	200638_s_at	*YWHAZ*	0.26	0.23		0.012
51	10q23.31	205235_s_at	*MPHOSPH1*		0.40	0.16	0.050

[†]Correlation between each gene's log-scale expression and the CKS1B amplification index (N = 197, all patients with both GEP and FISH 1q21). Blank cells correspond to correlations with P > 0.05.

[‡]Correlation between each gene's log-scale expression and CKS1B log-scale expression (N = 351, all patients with GEP). Rows with CKS1B |r| > = 0.4 are formatted bold.

[⋆]Correlation between each gene's log-scale expression and the PCLI (N = 305, 46 patients are missing PCLI).

[a]Multivariate proportional hazards regression of overall survival on extreme quartile expression (Q1 or Q4) for each gene, adjusted for FISH 13 80%, cytogenetic abnormalities, B2M > 4, CRP > 4, ALB < 3.5 and PCLI (N = 277, 74 patients are missing at least one measurement; see the supplemental methods for details).

Table Ib. Quartile 1 gene probe sets satisfying FDR 2.5% cutoff

Rank (Q1)	Chromosome	Probe set	Symbol	CKS1B Amplification Index r[†]	CKS1B r[‡]	PCLI r[⋆]	Adjusted Survival *P*-value[a]
1	9q31.3	201921_at	GNG10	−0.20	−0.30		0.600
2	1p13	227278_at	NA			−0.12	0.900
3	Xp22.3	209740_s_at	PNPLA4				0.029
4	20q11.21	227547_at	NA	−0.29	−0.28	−0.15	0.630
5	10q25.1	225582_at	KIAA1754	−0.21	−0.32		0.003
6	1p13.2	200850_s_at	AHCYL1			−0.13	0.019
7	1p13.3	213628_at	MCLC	−0.30	−0.28	−0.15	0.440
	1p22	209717_at	EVI5	−0.33	−0.29	−0.16	0.870
	1p13.3	222495_at	AD−020	−0.30	−0.24	−0.20	0.920
10	6p21.31	1557277_a_at	NA		−0.11		0.460
11	1p22.1	1554736_at	PARG1		−0.20	−0.11	0.280
12	1p22	218924_s_at	CTBS	−0.16	−0.11	−0.13	0.460
13	**9p13.2**	**226954_at**	**NA**	**−0.22**	**−0.40**		**0.090**
14	1p34	202838_at	FUCA1	−0.17	−0.23		0.066
15	13q14	230192_at	RFP2	−0.28	−0.18		0.880
16	12q13.11	48106_at	FLJ20489	−0.23	−0.23	−0.11	0.300
17	11q13.1	237964_at	NA	−0.16	−0.20		0.044
18	2p22-p21	202729_s_at	LTBP1	−0.24	−0.21		0.097
19	1p13.1	212435_at	NA	−0.21	−0.21	−0.11	0.034

[†]Correlation between each gene's log-scale expression and the CKS1B amplification index (N = 197, all patients with both GEP and FISH 1q21). Blank cells correspond to correlations with $P > 0.05$.

[‡]Correlation between each gene's log-scale expression and CKS1B log-scale expression (N = 351, all patients with GEP). Rows with CKS1B $|r| >= 0.4$ are formatted bold.

⋆Correlation between each gene's log-scale expression and the PCLI (N = 305, 46 patients are missing PCLI).

[a]Multivariate proportional hazards regression of overall survival on extreme quartile expression (Q1 or Q4) for each gene, adjusted for FISH 13 80%, cytogenetic abnormalities, B2M >4, CRP >4, ALB <3.5 and PCLI (N = 277, 74 patients are missing at least one measurement; see the Supplemental Methods for details).

>=4 copies at diagnosis, five retained >=4 copies at relapse. Multivariate proportional hazards analyses revealed that *CKS1B* amplification, chromosome 13q14 deletion, and metaphase karyotype abnormalities all independently conferred both inferior event-free and overall survival, whereas hypo-albuminemia was the only one of three standard prognostic factors that retained adverse implications for both endpoints examined. *CKS1B* amplification was an independent predictor of outcome both as a 0–100 scale index and as a two-group category after adjustment for other variables. In these analyses, a patient group with an 0–100 scale index one unit larger than another has an estimated 0.9% higher risk of progression and 1.1% higher risk of death (i.e. an increase of approximately 1% in risk with each increase of 1 in the index). The frequency of *CKS1B* quartile 4 expression varied among previously reported genetic subgroups [5]. With respect to gene expression-based identification of those with recurrent translocations, nearly two-thirds of patients with *MAF* or *MAFB* activation, one-third each with *FGFR3/MMSET* and *CCND1* activation, and only 18% of those without these translocations had *CKS1B* in quartile 4 ($P < 0.0001$). When examined in the context of metaphase karyotypes, *CKS1B* quartile 4 expression was present in approximately 20% of cases with hyperdiploid or normal, i.e. uninformative, karyotypes, whereas this feature was seen in nearly 50% of patients with hypodiploid and other cytogenetic abnormalities ($P = 0.0002$). In a separate multivariate analysis that adjusted for genetic subgroups, *CKS1B* quartile 4 expression remained an independent adverse outcome predictor; the gene expression-derived translocation

Table IIa. Multivariate proportional hazards analysis[†] (n = 369)

		Event-Free Survival			Survival		
	%	HR	P	Cumulative r^2	HR	P	Cumulative r^2
CKS1B Amplification Index 0–100)		1.009	0.002	0.160	1.011	0.002	0.219
FISH Chromosome 13 Deletion	25.5	1.786	0.006	0.224	1.879	0.014	0.308
Abnormal Karyotype	35.0	1.875	0.001	0.272	2.298	< 0.001	0.393
Beta-2-microglobulin >=4 mg/L	35.8	1.478	0.046	0.305	1.396	0.170	0.422
C-reactive protein >=4 mg/L	63.4	1.533	0.028	0.320	1.586	0.055	0.448
Albumin <3.5 g/dL	16.5	1.660	0.019	0.336	1.698	0.044	0.461
Events/Deaths		127			84		

Table IIb. Multivariate proportional hazards analysis[†] (n = 369)

		Event-Free Survival			Survival		
	%	HR	P	Cumulative r^2	HR	P	Cumulative r^2
CKS1B Amplification Index > =46	32.5	1.68	0.008	0.132	2.12	0.001	0.207
FISH Chromosome 13 Deletion	25.5	1.74	0.010	0.204	1.83	0.020	0.293
Abnormal Karyotype	35.0	1.94	< 0.001	0.257	2.33	< 0.001	0.383
Beta-2-microglobulin > =4 mg/L	35.8	1.52	0.033	0.293	1.43	0.140	0.417
C-reactive protein > = 4 mg/L	63.4	1.49	0.038	0.312	1.56	0.060	0.443
Albumin <3.5 g/dL	16.5	1.69	0.016	0.331	1.73	0.035	0.455
Events/Deaths	127				84		

[†]369 of 421 patients with CKS1B amplification measurements had complete measurements for this analysis; see the supplemental methods for details.

a) Multivariate proportional hazards analysis with the continuous CKS1B amplification index. A patient group with an index one unit larger than another has an estimated 0.9% higher risk of progression and 1.1% higher risk of death (i.e. an increase of approximately 1% in risk with each increase of 1 in the index). Labeling index was not significant in either analysis ($P > 0.35$, HR < 1.11, N = 325, EFS events = 116, deaths = 77, with 44 additional subjects missing the labeling index).

b) Multivariate proportional hazards analysis with a cutoff of > =46 for the CKS1B amplification index. Labeling index was not significant in either analysis ($P > 0.32$, HR < 1.12, N = 325).

category as a whole conferred inferior event-free ($P = 0.034$), but not inferior overall survival $P = 0.261$); however, consistent with published data [15,21], *CCND1* spikes impacted both endpoints favorably. While not adjusted for the multiple log rank tests that identified the 70 genes, this analysis, suggests that *CKS1B* expression retains predictive power in the context of genetic risk groups. Since CKS1 is required for SCF^{Skp2}-mediated ubiquitinylation and proteasomal degradation of $p27^{Kip1}$ [42,43], we next tested the relationship between CKS1 expression and $p27^{Kip1}$ in myeloma plasma cells. Western blot analysis of protein extracts from plasma cells from 27 newly diagnosed myeloma cases and 7 myeloma cell lines for which microarray data was also available, revealed a strong correlation between *CKS1B* mRNA

Supplemental Table 1. Chromosome distribution of 2.5% FDR gene probe sets

	U133Plus 2.0		Q1		Q4		Combined		
Chromosome	Number of Genes	%	Number of Genes	%	Number of Genes	%	Number of Genes	%	P value*
1	3,659	**9.9**	9	47.4	12	23.5	21	**30.0**	< 0.0001
2	2,522	6.9	1	5.3	2	3.9	3	4.3	
3	2,116	5.8	0	0.0	7	13.7	7	10.0	
4	1,456	4.0	0	0.0	0	0.0	0	0.0	
5	1,718	4.7	0	0.0	2	3.9	2	2.9	
6	2,005	5.4	1	5.3	1	2.0	2	2.9	
7	1,798	4.9	0	0.0	2	3.9	2	2.9	
8	1,311	3.6	0	0.0	4	7.8	4	5.7	
9	1,463	4.0	2	10.5	0	0.0	2	2.9	
10	1,444	3.9	1	5.3	2	3.9	3	4.3	
11	2,069	5.6	1	5.3	1	2.0	2	2.9	
12	1,927	5.2	1	5.3	3	5.9	4	5.7	
13	730	2.0	1	5.3	0	0.0	1	1.4	
14	1,195	3.2	0	0.0	0	0.0	0	0.0	
15	1,152	3.1	0	0.0	0	0.0	0	0.0	
16	1,507	4.1	0	0.0	2	3.9	2	2.9	
17	2,115	5.7	0	0.0	3	5.9	3	4.3	
18	582	1.6	0	0.0	1	2.0	1	1.4	
19	2,222	6.0	0	0.0	3	5.9	3	4.3	
20	1,072	2.9	1	5.3	2	3.9	3	4.3	
21	468	1.3	0	0.0	1	2.0	1	1.4	
22	906	2.5	0	0.0	1	2.0	1	1.4	
X	1,273	3.5	1	5.3	2	3.9	3	4.3	
Y	80	0.2	0	0.0	0	0.0	0	0.0	
m	5	0.0	0	0.0	0	0.0	0	0.0	
	36,795		19		51		70		
Unknown	17,880								
	54,675								

*An exact test for binomial proportions was used to compare the proportion of retained probe sets mapping to chromosome 1 to the proportion for the entire array.

Supplemental Table 2. Relationship between *CKS1B* gene expression quartiles and *CKS1B* amplification by interphase fluorescence in-situ hybridization in newly diagnosed myeloma

CKS1B Expression[†]	# Amplified	% Amplified
quartile 1[‡] n = 44	9	20%
quartile 2 n = 43	12	28%
quartile 3 n = 51	26	51%
quartile 4 n = 59	44	75%
total197	91	46%

[†]$P < 0.0001$. Amplification is defined as > = 20% of cells with 3 or > = 4 CKS1B signals, for validation in conjunction with Figure 2b–c, as described in the Methods. Other tables use the CKS1B amplification index and its optimal cutoff.
[‡]Quartile assignments based upon 351 patients with GEP

and protein, but no correlation between *CDKN1B (p27^{Kip1})* mRNA and protein levels. There was an inverse correlation between CKS1B and p27^{Kip1} protein levels. To confirm that CKS1B regulates p27^{Kip1} protein and possibly myeloma cell growth, we generated myeloma cell lines that constitutively express a small interfering RNA to *CKS1B*. Western blot analysis of the ARP1 myeloma cell line (containing 8 copies of *CKS1B*) transduced with lentivirus expressing iRNA to *CKS1B* or a scrambled iRNA revealed a marked reduction and increase in CKS1B and CDKN1B protein levels in CKS1B iRNA treated cells relative to control cells, respectively. A time course analysis of ARP1 cell growth also revealed a reduction in cell proliferation in CKS1B iRNA-positive cells relative to controls.

Global gene expression analysis of highly purified plasma cells from a large cohort of uniformly treated patients with myeloma identified 70 genes that were significantly correlated with early disease-related mortality. An unexpected feature of this list was that 30% of the 70 genes mapped to chromosome 1. Interestingly, all underexpressed genes from 1 were from 1p and 9% of the overexpressed genes were from chromsome 1qa feature consistent with cytogenetic and molecular cytogenetic data of frequent 1q gains and 1p losses in myeloma [18,21,28,29,44–47]. Tandem duplications and jumping translocations involving 1q21, caused by decondensation of pericentromeric heterochromatin [28,29,48], are cardinal features of progressive end-stage disease in myeloma suggesting that genes from this region may be driving myeloma progression.

DNA synthesis is mediated by the action of the cyclin E/CDK2 complex, which in turn is negatively regulated by the cyclin-dependent kinase inhibitor p27^{Kip1} [49]. The small evolutionarily conserved protein CKS1 is required for SCF^{Skp2}-mediated ubiquitination and proteasomal degradation of p27^{Kip1} [45,46], degradation of which permits DNA replication and correct progression of cells through S phase into mitosis [50]. Morris et al. recently showed that CKS proteins also interact with the proteasome to control the proteolysis of mitotic cyclins by way of regulating the transcriptional activity of *CDC20* [51], a regulatory subunit of the anaphase-promoting complex/cyclosome ubiquitin ligase [52]. Thus, CKS1 and the SCF^{Skp2}-CDKN1B-Cdk1/2 axis appear to be important for both DNA synthesis and mitosis [53]. The low p27^{Kip1} protein levels in cancer cells along with the conspicuous absence of inactivating gene mutations, has prompted speculation that hyperactivation of *CKS1B* and/or *SKP2*, may account for the low levels of p27^{Kip1} in cancer [54,55].

Recent studies have shown that loss of p27^{Kip1} is associated with shortened survival in patients with myeloma [56]. Based on the current data we propose that increased degradation of p27^{Kip1} and poor prognosis in myeloma is primarily caused by a gene-dosage related increase in *CKS1B* gene expression. In

Supplemental Table 3. Relationship of quartile 4 gene expression to amplification for genes located on bacterial artificial chromosome (BAC) used to measure 1q21 amplification

	Not Amplified		Amplified* (Amplification. Index. > = 46)				
Symbol	n/129	(%)	n/68	(%)	*P*-Value[†]	Amplification Index r[‡]	Log Rank *P*-Value[a]
PBXIP1	24	(18.6)	28	(41.2)	0.0012	0.29	0.5285
CKS1B	20	(15.5)	39	(57.4)	<0.0001	0.50	0.0002
PB591	23	(17.8)	38	(55.9)	<0.0001	0.43	0.0873
LENEP	31	(24.0)	18	(26.5)	0.8389	0.03	0.6507
ZFP67	27	(20.9)	29	(42.6)	0.0023	0.34	0.8717
FLJ32934	28	(21.7)	11	(16.2)	0.4606	−0.02	0.6207
ADAM15	23	(17.8)	29	(42.6)	0.0003	0.23	0.2808
EFNA4	26	(20.2)	23	(33.8)	0.0528	0.21	0.3212

The 0–100 scale CKS1B amplification index is a weighted sum of the proportions of clonal cells with 3 copies of CKS1B and > = 4 copies of CKS1B, defined by (.34% 3 copies + .66*% > = 4 copies)/.66
[†]For a test of the independence of amplification and 4th quartile membership (N = 197)
[‡]Correlation between each gene's expression and the 0–100 scale CKS1B amplification index
[a]Log rank test for association of Q4 membership and overall survival (N = 351, 64 deaths)

Supplemental Table 4 a. Relationship between genetic abnormalities and *CKS1B* expression in quartile 4

Abnormality		CKS1B Q4		
Category†	n/347 (%)	n	(%)	*P*-Value⋆
Expression-derived translocation				
t(11;14)	60 (17.3)	20	(33.3)	< 0.0001
t(4;14)	48 (13.8)	17	(35.4)	
t(14;16) & t(14;20)	14 (4.0)	9	(64.3)	
No Translocation Spike	225 (64.8)	41	(18.2)	
Metaphase karyotype				
Hyperdiploid	55 (15.9)	10	(18.2)	0.0002
Non-hyperdiploid	48 (13.8)	24	(50.0)	
Other Cytogenetics Abnormality	9 (2.6)	4	(44.4)	
No Cytogenetics Abnormality	235 (67.7)	49	(20.9)	
Chromosome 13 Deletion	n/334			
No	224 (67.1)	47	21.0	0.02
Yes	110 (32.9)	37	33.6	

†Translocations were determined from the expression spikes t(11;14) = CCND1, t(4:14) = FGFR3/MMSET, t(14;16) = MAF and t(14;20) = MAFB. Aneuploidy and other cytogenetic abnormalities were determined from cytogenetics, for which 4 observations were missing.

⋆Fisher's exact test of the independence of each category and CKS1B 4th quartile membership. Under the null hypothesis, Q4 contains on average 25% of patients within each level, corresponding to a proportional distribution across Q1-3 and Q4.

support of this concept we also observed that *CKS1B* over-expression and amplification commonly surfaced at relapse in patients lacking such features at diagnosis suggesting that tandem duplications and jumping translocations promote myeloma progression through amplification of the *CKS1B* gene.

CKS1B has been shown to be overexpressed in oral, gastric, colon, ovarian, and non-small cell lung carcinomas, and blastoid variant of mantle cell lymphoma [37,57–62] and its expression linked to poor survival in oral, gastric, and colorectal carcinomas [37,58,61]. Since 1q21 amplification and $p27^{Kip1}$ degradation is frequently observed in advanced malignancies, it will be important to determine whether *CKS1B* overexpression and disease progression/aggressiveness in other tumor types is linked to increased DNA copy numbers of *CKS1B*.

SKP2 overexpression has been observed in many tumor types and has been linked to poor survival in some cancers [63]. *SKP2* gene expression levels were not linked to survival in this analysis (data not shown) and we found that unlike CKS1B expression, *SKP2* expression was relatively high and not significantly different amongst plasma cells from normal healthy donors, MGUS, smoldering myeloma and myeloma (data not shown). Taken together these data suggests that CKS1B may be the rate-limiting component of the SCF^{Skp2} complex in myeloma cells.

In the current analysis we discovered that *CKS1B* overexpression was significantly linked to hypodi-

Supplemental Table 4 b. Multivariate analysis of CKS1B quartile 4 expression and cytogenetic abnormalities†

	Event-Free Survival		Survival	
	HR	P‡	HR	P‡
CKS1B Q4	1.97	0.003	2.16	0.005
Expression-derived translocation⋆				
t(11;14)	0.59	0.034	0.82	0.261
t(4;14)	1.67		1.77	
t(14;16) & t(14;20)	1.48		1.12	
Metaphase karyotype⋆⋆				
Hyperdiploid	1.75	0.006	1.84	0.013
Non-hyperdiploid	2.29		2.56	
Other Cytogenetics Abnormality	2.35		2.71	
r^2	0.218		0.223	
Events/Deaths	97		63	

†N = 347. Of 351 patients with expression data, 4 are missing cytogenetics.

‡Partial likelihood ratio test for the overall effect of the category.

⋆The *P*-value for modification of the CKS1B effect on EFS by translocation subgroup is 0.74

Supplemental Table 5. Overexpression CKS1B and CKS2 are related to ploidy.

CKSIB	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*p*-value
Hyperdiploidy (%)	66	61	27	28	2.64×10^{-7}
Hypodiploidy (%)	9	18	39	32	0.00099
Diploidy (%)	25	21	33	41	0.23
Total (N)	85	87	84	79	
CKS2	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*-value
Hyperdiploidy (%)	23	42	59	62	7.74×10^{-5}
Hypodiploidy (%)	40	23	17	14	0.0096
Diploidy (%)	37	42	24	25	0.25
Total (N)	86	86	82	81	

ploidy as determined metaphase cytogenetics. We have subsequently confirmed this relationship using DNA content analysis by flow cytometry in ~300 samples (unpublished data). Using gene expression profiling to identify molecular determinants of myeloma bone disease we recently showed that *CKS2* was one of only four genes significantly overexpressed in myelomas with osteolytic lesions [23]. Subsequently we noted that osteolytic lesions predominate in myelomas with hyperdiploid gene expression signatures [12]. Indeed, overexpression of *CKS2* was linked to hyperdiploid myeloma as determined by flow cytometry (unpublished data). Several studies have suggested a link between CKS genes in DNA ploidy. Hixon et al. showed that CKS1 mediates vascular smooth muscle cell polyploidization [64] and Spruck et al. revealed that CKS2 expression is required for the first metaphase/anaphase transition of mammalian meiosis [65]. Future studies will be aimed at determining if over-expression of either of the CKS family members can alter ploidy in myeloma cells.

CKS1B gene amplification along with chromosome 13q14 deletion and abnormal metaphase cytogenetics accounted for almost 40% of the observed survival variability inthis analysis, underscoring that myeloma risk is best assessed by molecular and cellular genetic tests. Routine application of such studies, performed on a single bone marrow sample, is recommended for appropriate patient stratification in therapeutic trial design. The survival impact of new agents, such as bortezomib and thalidomide and its derivatives, will be profound if their clinical efficacy also extends to genetically defined high-risk myeloma, which to date has not been investigated. CKS1B function appears to directly or indirectly interact with ubiquitin ligases and/or the proteasome to regulate cell cycle progression [66]. New therapeutic strategies that directly target CKS1B or related pathways may represent novel, and a more specific, means of treating de novo high-risk myeloma and may prevent secondary evolution.

Cyclin D dysegulation is a common event in cancer and contributes to tumorigenesis by promoting hyperphosphorylation of the RB1 protein, activation of E2F, and transition through the early G1 to S phase of the cell cycle. We have recently reported that dysregulated expression of one of the three D-type cyclins may be initiating genetic lesions in MM [12]. Based on data presented here we propose a two-hit pathogenetic model of myelomagenesis in which activation of a D type cyclin is followed by *CKS1B* amplification leading to dysregulation of both early and late G1 to S phases of the cell cycle.

Acknowledgements

We are indebted to members of the Donna D. and Donald M. Lambert Laboratory of Myeloma Genetics: Christopher Adams, Christopher Crane, Adam Hicks, Bob Kordsmeier, Ling Liu, Christopher Randolph, Steven McMorran, Owen Stephens, Rohit Ohja, Ryan Williams, Yan Xaio, and Hongwei Xu, for technical assistance. We extend a special thanks to all patients, their caregivers and referring physicians for making this work possible.

References

[1] Barlogie B, Shaughnessy J, Munshi N, Epstein J. Plasma cell myeloma. Williams Hematology 2001;1279–1304.
[2] Child JA, Morgan GJ, Davies FE, Owen RG, Bell SE, Hawkins K, Brown J, Drayson MT, Selby PJ. Medical Research Council Adult Leukaemia Working Party. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J Med 2003;348:1875–1883.
[3] Attal M, Harousseau JL, Facon T, et al. Single versus double autologous stem-cell transplantation for MM. N Engl J Med 2003;349:2495–2502.
[4] Barlogie B, Shaughnessy J, Tricot G, et al. Treatment of Multiple Myeloma. Blood 2004;103:20–32.
[5] Fonseca R, Barlogie B, Bataille R, et al. Genetics and cytogenetics of MM: a workshop report. Cancer Res 2004; 64:1546–1558.
[6] Smadja NV, Fruchart C, Isnard F, Louvet C, Dutel JL, Cheron N, Grange MJ, Monconduit M, Bastard C. Chromosomal analysis in multiple myeloma: cytogenetic evidence of two different diseases. Leukemia 1998;12:960–969.
[7] Kuehl WM, Bergsagel PL. MM: evolving genetic events and host interactions. Nature Rev Cancer 2002;2:175–187.
[8] Shaughnessy J, Barlogie B. Interpreting the molecular biology and clinical behavior of MM through global gene expression profiling. Immunol Rev 2003;94:140–163.
[9] Fonseca R, Debes-Marun CS, Picken EB, Dewald GW, Bryant SC, Winkler JM, Blood E, Oken MM, Santana-Davila

R, Gonzalez-Paz N, et al. The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma. Blood 2003;102:2562–2567.

[10] Smadja NV, Leroux D, Soulier J, Dumont S, Arnould C, Taviaux S, Taillemite JL, Bastard C. Further cytogenetic characterization of multiple myeloma confirms that 14q32 translocations are a very rare event in hyperdiploid cases. Genes Chromosomes Cancer 2003;38:234–239.

[11] Sawyer JR, Waldron JA, Jagannath S, Barlogie B. Cytogenetic findings in 200 patients with multiple myeloma. Cancer Genet Cytogenet 1995;82:41–49.

[12] Bergsagel P, Kuehl M, Zhan F, Sawyer J, Barlogie B, Shaughnessy J. Cyclin D dysregulation: an early and unifying pathogenic event in MM. Blood 2005;106:296–303.

[13] Wuilleme S, Robillard N, Lode L, Magrangeas F, Beris H, Harousseau JL, Proffitt J, Minvielle S, Avet-Loiseau H. Intergroupe Francophone de Myelome. Ploidy, as detected by fluorescence in situ hybridization, defines different subgroups in multiple myeloma. Leukemia 2005;19:275–278.

[14] Cremer FW, Bila J, Buck I, Kartal M, Hose D, Ittrich C, Benner A, Raab MS, Theil AC, Moos M, Goldschmidt H, Bartram CR, Jauch A. Delineation of distinct subgroups of multiple myeloma and a model for clonal evolution based on interphase cytogenetics. Genes Chromosomes Cancer 2005; [Epub ahead of print].

[15] Fonseca R, Blood E, Rue M, Harrington D, Oken MM, Kyle RA, Dewald GW, Van Ness B, Van Wier SA, Henderson KJ, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. Blood 2003;101:4569–4575.

[16] Soverini S, Cavo M, Cellini C, Terragna C, Zamagni E, Ruggeri D, Testoni N, Tosi P, De Vivo A, Amabile M, et al. Cyclin D1 overexpression is a favorable prognostic variable for newly diagnosed multiple myeloma patients treated with high-dose chemotherapy and single or double autologous transplantation. Blood 2003;102:1588–1594.

[17] Keats JJ, Reiman T, Maxwell CA, Taylor BJ, Larratt LM, Mant MJ, Belch AR, Pilarski L. In multiple myeloma, t(4;14)(p16;q32) is an adverse prognostic factor irrespective of FGFR3 expression. Blood 2003;101:1520–1529.

[18] Smadja NV, Bastard C, Brigaudeau C Leroux, Fruchart C. Hypodiploidy is a major prognostic factor in MM. Blood 2001;98:2229–2238.

[19] Fassas AB, Spencer T, Sawyer J, Zangari M, Lee CK, Anaissie E, Muwalla F, Morris C, Barlogie B, Tricot G. Both hypodiploidy and deletion of chromosome 13 independently confer poor prognosis in multiple myeloma. Br J Haematol 2002;118:1041–1047.

[20] Fonseca R, Harrington D, Oken MM, Dewald GW, Bailey RJ, Van Wier SA, Henderson KJ, Blood EA, Rajkumar SV, Kay NE, et al. Biological and prognostic significance of interphase fluorescence in situ hybridization detection of chromosome 13 abnormalities (delta13) in multiple myeloma: an eastern cooperative oncology group study. Cancer Res 2002;62: 715–720.

[21] Shaughnessy J, Jacobson J, Sawyer J, et al. Continuous absence of metaphase abnormalities especially of chromosome 13 and hypodiploidy assures long term survival in MM treated with total therapy i: interpreted in the context of gene expression profiling. Blood 2003;101:3849–3856.

[22] Gertz MA, Lacy MQ, Dispenzieri A, Greipp PR, Litzow MR, Henderson KJ, Van Wier SA, Ahmann GJ, Fonseca R. Clinical implications of t(11;14)(q13;q32), t(4;14)(p16.3;q32), and -17p13 in myeloma patients treated with high-dose therapy. Blood 2005.

[23] Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, Shaughnessy JD Jr. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med 2003;349:2483–94.

[24] Robbiani DF, Chesi M, Bergsagel PL. Bone lesions in molecular subtypes of multiple myeloma. N Engl J Med 2004;351:197–198.

[25] Fonseca R, Bailey RJ, Ahmann GJ, Rajkumar SV, Hoyer JD, Lust JA, Kyle RA, Gertz MA, Greipp PR, Dewald GW. Genomic abnormalities in monoclonal gammopathy of undetermined significance. Blood 2002;100:1417–1424.

[26] Kaufmann H, Ackermann J, Baldia C, Nosslinger T, Wieser R, Seidl S, Sagaster V, Gisslinger H, Jager U, Pfeilstocker M, et al. Both IGH translocations and chromosome 13q deletions are early events in monoclonal gammopathy of undetermined significance and do not evolve during transition to multiple myeloma. Leukemia 2004;18:1879–1882.

[27] Chng WJ, Van Wier SA, Ahmann GJ, Winkler JM, Jalal SM, Bergsagel PL, Chesi M, Trendle MC, Oken MM, Blood E, et al. A validated FISH trisomy index demonstrates the hyperdiploid and non-hyperdiploid dichotomy in MGUS. Blood 2005; [Epub ahead of print].

[28] Sawyer JR, Tricot G, Mattox S, Jagannath S, Barlogie B. Jumping translocations of chromosome 1q in multiple myeloma: evidence for a mechanism involving decondensation of pericentromeric heterochromatin. Blood 1998;91:1732–1741.

[29] Sawyer JR, Tricot G, Lukacs JL, Binz RL, Tian E, Barlogie B, Shaughnessy J Jr. Genomic instability in multiple myeloma: evidence for jumping segmental duplications of chromosome arm 1q. Genes Chromosomes Cancer 2005;42:95–106.

[30] Philip P, Drivsholm, Hansen NE, Jensen MK, Killmann S-A. Chromosomes and Survival in Multiple Myeloma. A Banding Study of 25 Cases. Cancer Genetics and Cytogenetics 1980;2: 243–257.

[31] Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D, Brown PO. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nat Genet 1999;23:41–46.

[32] Pollack JR, Sorlie T, Perou CM, Rees CA, Jeffrey SS, Lonning PE, Tibshirani R, Botstein D, Borresen-Dale AL, Brown PO. Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. Proc Natl Acad Sci U S A 2002;99:12963–12968.

[33] Heidenblad M, Lindgren D, Veltman JA, Jonson T, Mahlamaki EH, Gorunova L, van Kessel AG, Schoenmakers EF, Hoglund M. Microarray analyses reveal strong influence of DNA copy number alterations on the transcriptional patterns in pancreatic cancer: implications for the interpretation of genomic amplifications. Oncogene 2005;24:1794–1801.

[34] Shaughnessy J Jr, Tian E, Sawyer J, et al. Prognostic impact of cytogenetic and interphase fluorescence in situ hybridization-defined chromosome 13 deletion in MM: early results of total therapy II. Br J Haematol 2003;120:44–52.

[35] Zhan F, Hardin J, Bumm K, et al. Global gene expression profiling of MM, monoclonal gammopathy of undetermined significance and normal bone marrow plasma cells. Blood 2002;99:1745–1757.

[36] Shaughnessy J, Tian E, Sawyer J, et al. High incidence of chromosome 13 deletion in MM detected by multi-probe interphase FISH. Blood 2000;96:1505–1511.

[37] Kitajima S, KY, Ogawa I, Bashir T. et al. Role of Cks1 overexpression in oral squamous cell carcinomas: cooperation with Skp2 in promoting p27 degradation. Am J Pathol 165: 2147–2155.

[38] Zufferey R, Nagy D, et al. Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo. Nat Biotechnol 1997; 15:871–875.

[39] Storey JD, Tibshirani R. Statistical significance for genome-wise studies. Proc Natl Acad Sci 2003;100:9440–9445.

[40] O'Quigley J, Xu R. Explained variation in proportional hazards regression. In: Crowley J, editor. Handbook of

Statistics in Clinical Oncology. New York, NY: Marcel Dekker; 2001. pp 397–410.

[41] R Development Core Team. R. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2004. ISBN 3-900051-07-0, URL http://www.R-project.org.

[42] Ganoth D, Bornstein G, Ko TK, Larsen B, Tyers M, Pagano M, Hershko A. The cell-cycle regulatory protein Cks1 is required for SCFSkp2-mediated ubiquitinylation of p27. Nat Cell Biol 2001;3:321–324.

[43] Spruck C, Strohmaier H, Watson M, Smith AP, Ryan A, Krek TW, Reed SI. A CDK-independent function of mammalian Cks1: targeting of SCFSkp2 to the CDK inhibitor CDKN1B. Mol Cell 2001;7:639–650.

[44] Avet-Loiseau H, Andree-Ashley LE, Moore D 2nd, Mellerin MP, Feusner J, Bataille R, Pallavicini MG. Molecular cytogenetic abnormalities in multiple myeloma and plasma cell leukemia measured using comparative genomic hybridization. Genes Chromosomes Cancer 1997;19:124–133.

[45] Liebisch P, Viardot A, Bassermann N, Wendl C, Roth K, Goldschmidt H, Einsele H, Straka C, Stilgenbauer S, Dohner H, Bentz M. Value of comparative genomic hybridization and fluorescence in situ hybridization for molecular diagnostics in multiple myeloma. Br J Haematol 2003;122:193–201.

[46] Nilsson T, Hoglund M, Lenhoff S, et al. A pooled analysis of karyotypic patterns, breakpoints and imbalances in 783 cytogenetically abnormal mutiple myelomas reveals frequently involved chromosome segments as well as significant age- and sex-related differences. Br J Haematol 2003;120:960–969.

[47] Gutierrez NC, Garcia JL, Hernandez JM, Lumbreras E, Castellanos M, Rasillo A, Mateo G, Hernandez JM, Perez S, Orfao A, San Miguel JF. Prognostic and biologic significance of chromosomal imbalances assessed by comparative genomic hybridization in MM. Blood 2004;104:2661–2666.

[48] Le Baccon P, Leroux D, Dascalescu C, Duley S, Marais D, Esmenjaud E, Sotto JJ, Callanan M. Novel evidence of a role for chromosome 1 pericentric heterochromatin in the pathogenesis of B-cell lymphoma and multiple myeloma. Genes Chromosomes Cancer 2001;32:250–64.

[49] Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev 1999;13: 1501–1512.

[50] Nakayama K, Nagahama H, Minamishima YA, Miyake S, Ishida N, Hatakeyama S, Kitagawa M, Iemura S, Natsume T, Nakayama KI. Skp2-mediated degradation of p27 regulates progression into mitosis. Dev Cell 2004;6:661–672.

[51] Morris MC, Kaiser P, Rudyak S, Baskerville C, Watson MH, Reed SI. Cks1-dependent proteasome recruitment and activation of CDC20 transcription in budding yeast. Nature 2003; 423:1009–1013.

[52] Peters JM. The anaphase-promoting complex: proteolysis in mitosis and beyond. Mol Cell 2002;95:931–943.

[53] Pagano M. Control of DNA synthesis and mitosis by the Skp2-p27-Cdk1/2 axis. Mol Cell 2004;14:414–416.

[54] Slingerland J, Pagano M. Regulation of the cdk inhibitor p27 and its deregulation in cancer. J Cell Physiol 2000;183:10–17.

[55] Bartek J, Lukas J. p27 destruction: Cks1 pulls the trigger. Nat Cell Biol 2001;3:E95–E98.

[56] Filipits M, Pohl G, Stranzl T, Kaufmann H, Ackermann J, Gisslinger H, Greinix H, Chott A, Drach J. Low $p27^{Kip1}$ expression is an independent adverse prognostic factor in patients with multiple myeloma. Clin Cancer Res 2003;9: 820–826.

[57] Inui N, Kitagawa K, Miwa S, Hattori T, Chida K, Nakamura H, Kitagawa M. High expression of Cks1 in human non-small cell lung carcinomas. Biochem Biophys Res Commun 2003; 303:978–984.

[58] Masuda TA, Inoue H, Nishida K, Sonoda H, Yoshikawa Y, Kakeji Y, Utsunomiya T, Mori M. Cyclin-dependent kinase 1 gene expression is associated with poor prognosis in gastric carcinoma. Clin Cancer Res 2003;9:5693–5698.

[59] Shapira M, Ben-Izhak O, Bishara B, Futerman B, Minkov I, Krausz MM, Pagano M, Hershko DD. Alterations in the expression of the cell cycle regulatory protein cyclin kinase subunit 1 in colorectal carcinoma. Cancer 2004;100:1615–1621.

[60] de Vos S, Krug U, Hofmann WK, Pinkus GS, Swerdlow SH, Wachsman W, Grogan TM, Said JW, Koeffler HP. Cell cycle alterations in the blastoid variant of mantle cell lymphoma (MCL-BV) as detected by gene expression profiling of mantle cell lymphoma (MCL) and MCL-BV. Diagn Mol Pathol 2003;12:35–43.

[61] Shapira M, Ben-Izhak O, Linn S, Futerman B, Minkov I, Hershko DD. The prognostic impact of the ubiquitin ligase subunits Skp2 and Cks1 in colorectal carcinoma. Cancer 2005;103:1336–1346.

[62] Ouellet V, Provencher DM, Maugard CM, Le Page C, Ren F, Lussier C, Novak J, Ge B, Hudson TJ, Tonin PN, Mes-Masson AM. Discrimination between serous low malignant potential and invasive epithelial ovarian tumors using molecular profiling. Oncogene 2005;24:4672–4687.

[63] Bloom J, Pagano M. Deregulated degradation of the cdk inhibitor p27 and malignant transformation, Semin. Cancer Biol 2003;13:41–47.

[64] Hixon ML, Obejero-Paz C, Muro-Cacho C, Wagner MW, Millie E, Nagy J, Hassold TJ, Gualberto A. Cks1 mediates vascular smooth muscle cell polyploidization. J Biol Chem 2000;275:40434–40442.

[65] Spruck CH, de Miguel MP, Smith AP, Ryan A, Stein P, Schultz RM, Lincoln AJ, Donovan PJ, Reed SI. Requirement of Cks2 for the first metaphase/anaphase transition of mammalian meiosis. Science 2003;300:647–650.

[66] Pagano M, Benmaamar R. When protein destruction runs amok, malignancy is on the loose. Cancer Cell 2003;4:251–256.