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Cyclin D1 is frequently overexpressed in microsatellite unstable colorectal cancer, independent of CpG island methylator phenotype

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Abstract

Aims—Cyclin D1 and cyclin-dependent kinases are commonly activated in colorectal cancer. Microsatellite instability (MSI) and CpG island methylator phenotype (CIMP) are important molecular classifiers in colorectal cancer. However, the relationship between cyclin D1, MSI and CIMP has been uncertain.

Methods and Results—Among 865 colorectal cancers with MSI and CIMP data, 246 tumors (28.4%) showed cyclin D1 overexpression by immunohistochemistry. We quantified DNA methylation in p14 and 8 CIMP-specific promoters (*CACNA1G*, *CDKN2A* (p16), *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3* and *SOC1*) by real-time PCR (MethyLight). Both MSI-high and CIMP-high were associated with cyclin D1 overexpression ($p < 0.0001$). After tumors were stratified by MSI and CIMP status, the relation between MSI-high and cyclin D1 persisted ($p \leq 0.02$), whereas the relation between CIMP-high and cyclin D1 did not. Cyclin D1 overexpression was correlated with *BRAF* mutation ($p = 0.0001$), p27 loss ($p = 0.0007$) and p16 loss ($p = 0.02$), and inversely with p53 expression ($p = 0.0002$) and p21 loss ($p < 0.0001$). After stratification by MSI status, the inverse relation between cyclin D1 and p21 loss still persisted ($p < 0.008$).

Conclusions—cyclin D1 activation is associated with MSI and inversely with p21 loss in colorectal cancers. Cyclin D1 may play an important role in the development of MSI-high tumors, independent of CIMP status.

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Keywords

colon cancer; CCND1; CpG island methylator phenotype; CIMP; microsatellite instability; MSI; p21; CDKN1A; cyclin; DNA methylation

Introduction

Cyclin D1 plays a key role in cell cycle control, particularly in the transition from G1 to S phase, which is regulated by cyclin-dependent kinases.¹ Cyclin D1 overexpression (at both mRNA and protein levels) occurs in one-third or more of colorectal cancers.^{2–4} The ability of cyclin D1 to drive the cell cycle forward can be blocked by cyclin-dependent kinase (CDK) inhibitors, such as p21 (*CDKN1A*) and p27 (*CDKN1B*).¹ In addition, cyclin D1 is a target gene of the WNT/ β -catenin (*CTNNB1*) pathway. It has been suggested that cyclin D1 transactivation secondary to APC or β -catenin mutations participates in colon cancer initiation.^{5,6} Molecular correlates with cyclin D1 activation are important in understanding carcinogenic mechanisms in various molecular subtypes of colorectal cancer. However no study has comprehensively examined the relationship between cyclin D1, CDK inhibitors and microsatellite instability (MSI) in colorectal cancer.

Epigenetic aberrations are important mechanisms in human carcinogenesis.⁷ A number of tumor suppressor genes are silenced by promoter methylation during colorectal cancer development,⁸ and a subset of colorectal cancers exhibit widespread promoter methylation, which is referred to as the CpG island methylator phenotype (CIMP).⁹ CIMP-high colorectal tumors have distinct features, such as associations with proximal tumor location, female gender, *BRAF* mutation¹⁰ and wild-type *TP53*, independent of MSI status.^{11,12} In addition, CIMP-high is associated with intact p21 expression and loss of p27, independent of MSI.^{13,14} A molecular classification based on MSI and CIMP status is increasingly important,¹⁵ because MSI and CIMP reflect global genomic and epigenomic aberrations, respectively, in tumor cells.¹⁶

In this study, using quantitative DNA methylation analysis (MethyLight technology) and a large number of population-based colorectal cancers, we examined cyclin D1 expression, in relation to various clinicopathologic and molecular features, including expressions of CDK inhibitors (p21, p27 and p16), MSI and CIMP status. We have found that cyclin D1 overexpression is common in MSI-high tumors with intact p21 expression.

Materials and methods

Study group

We utilized the databases of two large prospective cohort studies; the Nurses' Health Study (N = 121,700 women followed since 1976),^{17,18} and the Health Professional Follow-up Study (N = 51,500 men followed since 1986).¹⁸ Informed consent was obtained from all participants who returned initial questionnaires and subsequently developed colorectal cancer. A subset of the cohort participants developed colorectal cancers during prospective follow-up. Thus, these colorectal cancers represented population-based, relatively unbiased samples (compared to retrospective or single-hospital-based samples). Previous studies on the Nurses' Health Study and Health Professionals Follow-up Study have described baseline characteristics of cohort participants and incident colorectal cancer cases, and confirmed that our colorectal cancers were well representative as a population-based sample.^{17,18} Follow-up of these cohorts are ongoing. Although survival data have not yet been available, it will be available in the future. We collected paraffin-embedded tissue blocks from hospitals where cohort participants with colorectal cancers had undergone resections of primary tumors. Based on availability of

adequate tissue specimens, a total of 865 colorectal cancers (377 from men's cohort and 488 from women's cohort) were included (the mean age at diagnosis, 66.2 years old with standard deviation 8.4). Among our cohort studies, there was no significant difference in demographic features between cases with tissue available and those without available tissue.¹⁸ Many of the cases have been previously characterized for statuses of CIMP, MSI, p21, p27 and p53.^{13,14} However, we have not examined cyclin D1 expression in our tumors. Tissue collection and analyses were approved by the Dana-Farber/Harvard Cancer Center and Brigham and Women's Hospital Institutional Review Boards.

Histopathologic evaluations

Hematoxylin and eosin (H&E) stained tissue sections were examined by a pathologist (S.O.) blinded from clinical and other laboratory data. The following features were evaluated. (1) The degree of tumor differentiation was categorized as well/moderate ($\geq 50\%$ gland formation) vs. poor ($< 50\%$ gland formation). (2) The presence and extent of extracellular mucin were categorized as 0% (no mucin), 1–49% or $\geq 50\%$ of the tumor volume. (3) The presence and extent of signet ring cells were categorized as 0% (no signet ring cells), 1–49% or $\geq 50\%$ of the tumor volume.

Genomic DNA extraction and sequencing of *KRAS* and *BRAF*

Genomic DNA was extracted from dissected tumor tissue sections.¹⁹ Normal DNA was obtained from colonic tissue at resection margins. Whole genome amplification (WGA) of genomic DNA was performed by PCR using random 15-mer primers.¹⁹ PCR and Pyrosequencing targeted for *KRAS* codons 12 and 13,¹⁹ and *BRAF* codon 600 were performed as previously described.²⁰

Analyses for microsatellite instability (MSI) and *TGFBR2* and *BAX* mutations

MSI analysis was performed as previously described.²¹ In addition to D2S123, D5S346, D17S250, BAT25 and BAT26,²² we used BAT40, D18S55, D18S56, D18S67 and D18S487 (i.e., 10-marker panel).²¹ A “high degree of MSI” (MSI-H) was defined as the presence of instability in $\geq 30\%$ of the markers. A low degree of MSI (MSI-L) was defined as instability in $< 30\%$ of the markers, and “microsatellite stable (MSS)” tumors were defined as tumors without an unstable marker. Methods to detect mutations in *TGFBR2*²³ and *BAX* mononucleotide repeats have been previously described.²⁴

Real-time PCR (MethyLight) for quantitative DNA methylation analysis

Sodium bisulfite treatment on genomic DNA and subsequent real-time PCR (MethyLight²⁵) were validated and performed as previously described.²⁶ We quantified DNA methylation in 8 CIMP-specific promoters (*CACNA1G*, *CDKN2A* (p16), *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3* and *SOC3*),²⁷ all of which were selected from screening of 195 CpG islands in the human genome.^{10,12} Primers and probes were previously described.¹⁰ In addition, we quantified p14 (*CDKN2A/ARF*) methylation with primers and probe; P14-F, 5'-TTGGAGGCGGCGAGAATAT-3' (Genbank No. L41934, nucleotide Nos. 238–256); P14-R, 5'-CCCCGTAAACCGCGAAATA-3' (nucleotide Nos. 332–350); P14-probe, 6FAM-5'-CGGTTCTGTCGCGAGTGAGGGTT-3'-BHQ-1 (nucleotide Nos. 299–320). The PCR condition was initial denaturation at 95°C for 10 min followed by 45 cycles of 95°C for 15 sec and 60°C for 1 min, and performed as previously described.²⁴

CIMP-high was defined as the presence of ≥ 6 of 8 methylated promoters (excluding p14), CIMP-low as the presence of 1/8–5/8 methylated promoters, and CIMP-0 as the absence (0/8) of methylated promoters, according to the previously established criteria.²⁷

Tissue microarrays (TMAs) and immunohistochemistry for p53, p21, p27, p16, β -catenin and cyclin D1

TMAs were constructed as previously described.²⁸ We examined two to four tumor tissue cores for each marker. We examined whole tissue sections in all cases (for p21, p27 and p16), and in cases (for other markers) in which no tissue block was available for TMAs or results were equivocal in TMAs. Methods for immunohistochemistry were previously described; p21,²⁹ p27,²⁹ p16,²¹ p53,¹⁴ and β -catenin.³⁰ For p53, moderate/strong nuclear staining in $\geq 50\%$ of tumor cells was interpreted as positive. For p21 and p27, nuclear staining in $< 5\%$ and $< 10\%$ of tumor cells, respectively, was interpreted as protein loss.

For cyclin D1, antigen retrieval was performed, and deparaffinized tissue sections in Target Retrieval Solution (Dako, Carpinteria, CA) were treated with microwave in a pressure cooker for 15 min. Tissue sections were incubated with 3% H₂O₂ (10 min) to block endogenous peroxidase, and with 10% normal goat serum (Vector Laboratories, Burlingame, CA) in phosphate-buffered saline (10 min). Primary antibody against cyclin D1 (rabbit monoclonal [SP4] to cyclin D1, 1:100 dilution; Abcam, Cambridge, MA) was applied, and the slides were maintained overnight at room temperature. Next, we applied an anti-rabbit IgG antibody (Vector Laboratories) for 30 min, followed by an avidin–biotin complex conjugate (Vector Laboratories) for 30 min. The immunochemical reaction was revealed by diaminobenzidine (5 min) and methyl-green counterstain. Nuclear cyclin D1 expression was recorded as no expression, weak expression, or moderate/strong expression. Cyclin D1 positivity (i.e., overexpression) was defined as $\geq 50\%$ of tumor cells with weak nuclear staining or $\geq 20\%$ of tumor cells with moderate/strong nuclear staining. The antigen retrieval procedure for examination of cyclin D1 was considered to be uniform across samples by virtue of the large number of cases, and we could secure enough number of cases in various strata of expression level (for example, expression in $< 10\%$ of tumor cells, 10–30% of tumor cells, 30–50% of tumor cells, $\geq 50\%$ of tumor cells, etc.). Then, we could see where the optimal cutoff was. Thus, our cut off for Cyclin D1 was not totally arbitrary. In any case any random misclassification would drive the results towards the null hypothesis of no difference in the frequencies of cyclin D1 overexpression. Despite such a possible misclassification, we still obtained statistically very significant results for many of the correlations.

Appropriate positive and negative controls were included in each run of immunohistochemistry. All immunohistochemically-stained slides were interpreted by a pathologist (S.O., except for cyclin D1 and β -catenin by K.N.) blinded from any other clinical and laboratory data.

Statistical analysis

For categorical data, chi-square test (or Fisher's exact test when any expected cell count was less than 5) was performed and odds ratio (OR) with 95% confidence interval (CI) was computed, using SAS program (Version 9.1, SAS Institute, Cary, NC). All p values were two-sided, and statistical significance was set at $p \leq 0.05$.

Results

Cyclin D1 overexpression in colorectal cancers

Among the 865 colorectal cancers that we examined cyclin D1 expression by immunohistochemistry, 246 (28.4%) tumors overexpressed cyclin D1 (Figure 1). Table 1 summarizes the frequencies of cyclin D1 overexpression in relation to various clinical and pathologic features. Cyclin D1 overexpression was more common in proximal tumors (34% = 117/343) than distal tumors (excluding rectum) (24% = 62/252, $p = 0.009$) and rectal tumors

(21%=36/169, $p=0.003$). However, after tumors were stratified by MSI status, cyclin D1 was no longer correlated with tumor location (data not shown).

Cyclin D1 overexpression was less frequent $\geq 50\%$ signet ring cell carcinoma (0%=0/15, $p=0.01$) than carcinoma with no signet ring cells (30%=194/644). However, after the tumors were stratified by MSI status, the correlation of cyclin D1 with signet ring cells did not persist (data not shown). Cyclin D1 was not correlated with any of the other clinical or pathologic features examined.

Cyclin D1 overexpression is associated with MSI-H, CIMP-high and BRAF mutation

Table 2 summarizes the frequencies of cyclin D1 overexpression in relation to molecular alterations in colorectal cancer. Cyclin D1 overexpression was significantly more common in microsatellite instability-high (MSI-H) tumors (54%=63/117, $p<0.0001$) than in microsatellite stable (MSS) tumors (24%=156/640). We determined CpG island methylator phenotype (CIMP) status using MethyLight assays on a panel of 8 CIMP-specific promoters (*CACNA1G*, *CDKN2A*, *CRABP1*, *IGF2*, *MLH1*, *NEUROG1*, *RUNX3* and *SOC3*)^{10,27}. Cyclin D1 overexpression was more common in CIMP-high tumors (48%=57/118, $p<0.0001$) than in CIMP-0 tumors (21%=81/384). Cyclin D1 overexpression was more common in *BRAF*-mutated tumors (44%=46/105, $p=0.0001$) than *BRAF* wild-type tumors (26%=184/715).

The relationship between cyclin D1 overexpression and MSI is independent of CIMP status

In order to examine effect of MSI (or CIMP) on cyclin D1 expression independent of CIMP (or MSI) status, we classified tumors into 4 subtypes according to combined MSI and CIMP status (Figure 2). The relationship between cyclin D1 overexpression and MSI-H persisted ($p\leq 0.02$) after stratification by CIMP status. In contrast, the relationship between CIMP and cyclin D1 expression did not persist after stratification by MSI status.

Cyclin D1 overexpression, MSI, and BRAF and KRAS mutation

To examine the interrelationship between MSI, *BRAF*, *KRAS* and cyclin D1, we stratified tumors according to combined MSI, *BRAF* and *KRAS* status (Figure 3). No significant relationship between *BRAF* mutation and cyclin D1 was present in MSI-H. However, among the MSI-L/MSS group, cyclin D1 was more commonly overexpressed in *BRAF*-mutated tumors than in *KRAS/BRAF*-wild-type tumors ($p=0.003$).

Cyclin D1 overexpression is inversely associated with p21 loss, independent of MSI status

We examined the relation between cyclin D1 and various cyclin-dependent kinase (CDK) inhibitors in colorectal cancer, because cyclin D1 is regulated by various CDK inhibitors (Figure 4). Cyclin D1 overexpression was significantly associated with p27 loss ($p=0.0007$), p16 loss ($p=0.02$), *CDKN2A* (p16) methylation ($p=0.0002$) and p14 methylation ($p<0.0001$), and inversely with p21 loss ($p<0.0001$) and p53 expression ($p=0.0002$) (Table 2). Considering the relationship between cyclin D1 and MSI (shown above) and the associations of MSI with p53, p21, p27 and p16,^{13,14,27} we stratified tumors by MSI status. The inverse relationship between p21 loss and cyclin D1 overexpression persisted ($p<0.008$) after stratification by MSI status (Figure 5). In contrast, the association of cyclin D1 with p27 loss, p16 loss, *CDKN2A* (p16) methylation, p14 methylation or p53 expression did not persist after stratification by MSI status (data not shown). We also stratified tumors by p53 and p21 status, because p21 is known to be induced by wild-type p53.³¹ It was evident that p53 status had no effect on cyclin D1 after tumors were stratified by p53 and p21 (Figure 6).

Cyclin D1 and other molecular changes

Cyclin D1 was not significantly associated with nuclear β -catenin. Among MSI-high tumors, cyclin D1 was not significantly associated with mutation in *TGFB2* or *BAX* (Table 2), in agreement with a previous study on cyclin D1 and *TGFB2*.³²

Discussion

We conducted this study to examine the relationship between cyclin D1 overexpression and various molecular alterations in colorectal cancers. Molecular correlates with abnormalities of cyclins, cyclin-dependent kinases (CDK) and CDK inhibitors are important in understanding carcinogenic mechanisms in various subtypes of colorectal cancer.^{13,14,32,33} In addition, a molecular classification based on MSI and CIMP status is increasingly important,¹⁵ because MSI and CIMP reflect global genomic and epigenomic aberrations, respectively, in tumor cells.¹⁶ We have demonstrated that cyclin D1 overexpression is associated with MSI-H and inversely with p21 loss, independent of CIMP status. Our data suggest that cyclin D1 may play an important role in the development of MSI-H tumors, especially when p21 is not down-regulated.

Our resource of a large number of colorectal cancers derived from the two prospective cohort studies has enabled us to precisely estimate the frequency of colorectal cancers with a specific molecular feature (such as cyclin D1 overexpression, MSI-H, etc.) at a population level. The large number of cases has also provided a sufficient power for rare subtypes of colorectal cancer such as MSI-H CIMP-low/0. For quantitative DNA methylation analysis on p14 and 8 CIMP-specific promoters including *CDKN2A* (p16), we have utilized MethyLight technology, which is robust and can reproducibly differentiate low-level methylation from high-level methylation.^{26,34}

Cyclin D1 (*CCND1*, the official gene symbol) activation has been implicated in carcinogenesis,¹ among many other molecular changes in colorectal cancer.^{35–39} Previous studies have reported that cyclin D1 overexpression occurs in 30–40% of colorectal cancers and adenomas,^{3,40} but not in hyperplastic polyps or normal mucosa.³ *CCND1* mRNA is overexpressed in colon cancer cell lines, but not in normal cells.⁴¹ A common polymorphism (so-called 870G>A) of *CCND1* appears to modify risks of colorectal cancer and adenoma^{42,43} and the age of onset of hereditary nonpolyposis colorectal cancer (HNPCC),⁴⁴ although other studies have shown no association of the *CCND1* polymorphism with age of onset of HNPCC.^{45,46} Relative amounts of *CCND1* transcript variants in blood/normal mucosa appear to modify the age of onset of HNPCC.⁴⁵ In addition to the pivotal role of cyclin D1 in cell cycle progression,¹ these epidemiologic data collectively support the importance of *CCND1* in colorectal carcinogenesis.

The inverse correlation between p21 down-regulation and cyclin D1 overexpression has been reported in colorectal cancers;^{40,47} however, MSI or CIMP status has not been examined in these studies. Previous studies have shown that p21 loss is inversely correlated with MSI-H in colorectal cancers,^{13,33} and we have shown that MSI-H is correlated with cyclin D1 overexpression (in this study). Thus, to support a pathogenetic interaction between p21 and cyclin D1, one must examine p21 and cyclin D1 in relation to MSI status. In the current study, we have indeed shown the inverse correlation between p21 loss and cyclin D1 overexpression independent of MSI status. Our data support a possible pathogenetic interaction between p21 and cyclin D1.

We have also demonstrated that the associations between cyclin D1 overexpression and various pathologic features (signet ring cell component, and tumor-infiltrating lymphocytes) appear to be mediated through MSI. Our findings seem to be reasonable. MSI status reflects genomic

aberrations in tumor cells and determines molecular features and phenotypes of colorectal cancer. Both pathologic features and cyclin D1 expression are likely influenced by genomic status of tumor cells. A previous study on endometrial carcinoma has also reported the relation between cyclin D1 overexpression and MSI.⁴⁸ Concerning with colorectal cancers, a recent study has reported that cyclin D1 expressions were generally low in 11 MSI-high tumors and higher expression levels were noted in 37 MSS/MSI-low tumors.⁴⁹ This discrepancy might be due to differences in the sample sizes and the methods and criteria for cyclin D1 interpretation.

In conclusion, cyclin D1 overexpression is associated with MSI and inversely with p21 down-regulation, independent of CIMP status in colorectal cancer. Our data imply that cyclin D1 may play an important role in the development of MSI-H tumors. An exact mechanism of the possible pathogenetic link between cyclin D1 activation and MSI remains to be investigated.

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Abbreviations and HUGO Gene Nomenclature Committee (HGNC)-approved official gene symbols

CCND1, cyclin D1
 CDK, cyclin-dependent kinase
 CDKN1A, cyclin-dependent kinase inhibitor 1A (p21/CIP1)
 CDKN1B, cyclin-dependent kinase inhibitor 1B (p27/KIP1)
 CDKN2A, cyclin-dependent kinase inhibitor 2A (p16/INK4A)
 CI, confidence interval
 CIMP, CpG island methylator phenotype
 CTNNB1, catenin (cadherin-associated protein), beta 1 (β -catenin)
 MSI, microsatellite instability
 MSS, microsatellite stable
 OR, odds ratio
 TGF, transforming growth factor
 TGFBR2, transforming growth factor- β receptor type 2
 TMA, tissue microarray

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Figure 1.

Cyclin D1 and p21 expressions in colorectal cancer.

A. Heterogeneous nuclear cyclin D1 expression in carcinoma (arrowheads) and very weak cyclin D1 expression in normal colonic mucosa (empty arrows). B. Strong and diffuse nuclear cyclin D1 overexpression in carcinoma (arrow). C. Loss of nuclear p21 in carcinoma (empty arrows). Inflammatory cells serve as internal positive control (solid arrowhead). D. Intact nuclear p21 expression in carcinoma cells (arrows).

Figure 2.

Frequency of cyclin D1 overexpression in four MSI/CIMP subtypes of colorectal cancer. The relationship between MSI and cyclin D1 overexpression is independent of CIMP status. CI, confidence interval; CIMP, CpG island methylator phenotype; MSI, microsatellite instability; OR, odds ratio.

Figure 3.

Frequency of cyclin D1 overexpression in colorectal cancer according to MSI, *KRAS* (K), and *BRAF* (B) statuses.

The relationship between *BRAF* mutation and cyclin D1 does not persist in MSI-H tumors.

Figure 4.

Cyclin D1, cyclin-dependent kinase (CDK) inhibitors (p21, p27, p16 and p14), p53 and the cell cycle pathway.

Note the close relationship between cyclin D1 and p21.

Figure 5.

Frequency of cyclin D1 overexpression in colorectal cancer according to MSI and p21 statuses. The inverse relationship between p21 loss and cyclin D1 overexpression is independent of MSI status. The relation between MSI and cyclin D1 somewhat persists after stratification by p21 status. Within p21 (1+) tumors, cyclin D1(+) is higher in MSI-H tumors (55%) than MSI-L/MSS tumors (34%, $p=0.007$), and within p21 (2+) tumors, cyclin D1(+) is higher in MSI-H (72%) than in MSI-L/MSS (52%, $p=0.06$).

Figure 6.

Frequency of cyclin D1 overexpression in colorectal cancer according to p53 and p21 statuses. The inverse relationship between p21 loss and cyclin D1 overexpression is independent of p53 status, while p53 is not related to cyclin D1 after stratification by p21 status.

Table 1
Frequency of cyclin D1 overexpression in colorectal cancer according to various clinical and pathologic features

Clinical/pathologic feature	Total N	Cyclin D1(+)	OR (95% CI)	P value
All cases	865	246 (28%)		
Gender				
Men	377	102 (27%)	1	
Women	488	144 (30%)	1.13 (0.84–1.52)	
Age				
≤59	185	48 (26%)	1	
60–69	326	99 (30%)	1.24 (0.83–1.87)	
≥70	267	70 (26%)	1.01 (0.66–1.55)	
Location*				Referent
Proximal	343	117 (34%)	1	
Distal (not Rectum)	256	62 (24%)	0.62 (0.43–0.89)	0.009
Rectum	169	36 (21%)	0.52 (0.34–0.80)	0.003
Stage				
I	177	47 (27%)	1	
II	234	77 (33%)	1.36 (0.88–2.09)	
III	208	52 (25%)	0.92 (0.58–1.46)	
IV	92	21 (23%)	0.82 (0.45–1.48)	
Tumor differentiation				
Well/moderate	747	209 (28%)	1	
Poor	79	23 (29%)	1.06 (0.63–1.76)	
Mucinous component				
0%	463	128 (28%)	1	
1–49%	175	60 (34%)	1.37 (0.94–1.98)	
≥50%	110	34 (31%)	1.17 (0.74–1.84)	
Signet ring cell				Referent
Component				
0%	644	194 (30%)	1	
1–49%	45	14 (31%)	1.05 (0.55–2.01)	
≥50%	15	0 (0%)	0 [^]	0.01

Only significant p values are described.

* Proximal colon includes cecum to transverse colon, and distal colon (not rectum) includes splenic flexure to sigmoid colon.

[^] Because of the presence of 0 cases in a category, 95% CI was not calculated. CI, confidence interval; OR, odds ratio.

Table 2
Frequency of cyclin D1 overexpression in colorectal cancer according to various molecular features

Molecular feature	Total N	Cyclin D1(+)	OR (95% CI)	P value
MSI status	640	156 (24%)	1	Referent
MSI-low	69	14 (20%)	0.79 (0.43–1.46)	
MSI-high	117	63 (54%)	3.62 (2.41–5.43)	<0.0001
CIMP status	384	81 (21%)	1	Referent
CIMP-0	301	86 (29%)	1.50 (1.05–2.12)	0.02
CIMP-low	118	57 (48%)	3.50 (2.26–5.41)	<0.0001
CIMP-high	532	178 (33%)	1	
KRAS mutation	302	86 (28%)	0.79 (0.58–1.08)	
+	715	184 (26%)	1	Referent
BRAF mutation	105	46 (44%)	2.25 (1.48–3.43)	0.0001
+	474	157 (33%)	1	Referent
p53 *	377	82 (22%)	0.56 (0.41–0.77)	0.0002
+	89	54 (61%)	1	Referent
p21 (CDKN1A) *	220	95 (43%)	0.54 (0.32–0.89)	0.01
2+ (intact)	479	84 (18%)	0.14 (0.085–0.22)	<0.0001
1+	221	46 (21%)	1	Referent
p27 (CDKN1B) *	471	157 (33%)	1.90 (1.30–2.77)	0.0007
Loss	432	107 (25%)	1	Referent
+	98	36 (37%)	1.76 (1.11–2.81)	0.02
p16 expression *	598	146 (24%)	1	Referent
Loss	246	91 (37%)	1.82 (1.32–2.50)	0.0002
CDKN2A (p16)	654	162 (25%)	1	Referent
methylation	163	67 (41%)	2.12 (1.48–3.04)	<0.0001
p14 (CDKN2A/ARF)	335	100 (29.9%)	1	
methylation	417	120 (28.8%)	0.95 (0.69–1.30)	
Nuclear β-catenin *	44	20 (45%)	1	
+	90	46 (51%)	1.25 (0.61–2.59)	
TGFBR2	101	45 (45%)	1	
mononucleotide mutation (only MSI-H tumors)				
BAX				

Molecular feature	Total N	Cyclin D1(+)	OR (95% CI)	P value
Mononucleotide mutation (only MSI-H tumors)	33	21 (64%)	2.18 (0.97–4.90)	

Only significant p values are described.

* p53, p21, p27, p16 and β -catenin statuses were determined by immunohistochemistry.

CI, confidence interval; CIMP, CpG island methylator phenotype; MSI, microsatellite instability; OR, odds ratio.