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CDK8 Expression in 470 Colorectal Cancers in Relation to β-Catenin Activation, Other Molecular Alterations and Patient Survival

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Abstract

Alterations in the Wnt/β-catenin pathway define a key event in the pathogenesis of colon cancer. We have recently shown that *CDK8*, the gene encoding a cyclin-dependent kinase (CDK) component of the Mediator complex, acts as a colon cancer oncogene that is necessary for β-catenin activity. Here, we tested the hypothesis that colorectal cancers with CDK8 expression have distinct clinical, prognostic and molecular attributes. Among 470 colorectal cancers identified in two prospective cohort studies, CDK8 expression was detected in 329 (70%) tumors by immunohistochemistry. Cox proportional hazards model and backward stepwise elimination were used to compute hazard ratio (HR) of deaths according to CDK8 status, initially adjusted for various patient and molecular features, including β-catenin, p53, p21, p27 (CDK inhibitors), cyclin D1, fatty acid synthase (FASN), cyclooxygenase-2 (COX-2), microsatellite instability (MSI), CpG island methylator phenotype (CIMP), LINE-1 methylation, and mutations in *KRAS, BRAF* and *PIK3CA*. CDK8 expression in colorectal cancer was independently associated with β-catenin activation (p=0.0002), female gender (p<0.0001) and FASN overexpression (p=0.0003). Among colon cancer patients, CDK8 expression significantly increased colon cancer-specific mortality in both univariate analysis [HR 1.70; 95% confidence interval (CI), 1.03-2.83; p=0.039] and multivariate analysis (adjusted HR 2.05; 95% CI, 1.18-3.56; p=0.011) that was adjusted for potential confounders including β-catenin, COX-2, FASN, LINE-1 hypomethylation, CIMP and MSI. CDK8 expression was unrelated with clinical outcome among rectal cancer patients. These

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data support a potential link between CDK8 and β-catenin, and suggest that CDK8 may identify a subset of colon cancer patients with a poor prognosis.

Keywords

colon cancer; CDK8; prognosis; CTNNB1; FASN

Introduction

Activation of the Wnt/β-catenin pathway occurs in the majority of colon cancers, making this pathway a particularly attractive therapeutic target.[1, 2] Despite extensive efforts to modulate this pathway, β-catenin itself has proven to be recalcitrant to small molecule antagonists.[3] To identify therapeutically amenable oncogenes that impinge on the Wnt/β-catenin pathway, we and others have used forward and reverse genetic approaches and have identified *CDK8* as a colon cancer oncogene necessary for β-catenin activity.[4, 5] CDK8 is a cyclin-dependent kinase (CDK) member of the mediator complex that couples transcriptional regulators to the basal transcriptional machinery,[6] and is implicated in the transcriptional regulation of key pathways involved in colon cancers such as Wnt/β-catenin,[7] Notch,[8] and p53.[9] The kinase activity of CDK8 is required for its ability to regulate β-catenin dependent transcription and oncogenesis.[4, 5] These observations suggest that therapeutic interventions that target the CDK8 kinase activity in colon cancers may be of clinical value.

To determine the molecular and clinical features of CDK8-expressing colorectal cancers, we have conducted a population-based study, using a large number (N=470) of colorectal cancers. We concurrently examined molecular alterations previously associated with colorectal cancer pathogenesis including *KRAS*, *BRAF* and *PIK3CA* mutations and microsatellite instability (MSI), as well as a number of other markers including β-catenin, fatty acid synthase (FASN), p53, p21, p27 and cyclin D1. These changes in colorectal cancer are related with each other, and potential confounders in survival analysis. We found that CDK8 expression was independently associated with β-catenin activation. Moreover, CDK8 expression was significantly associated with poor prognosis in colon cancer. These data define CDK8 expression in colorectal cancer as a biomarker with potentially important therapeutic implications.

Materials and Methods

Study Population

We utilized the databases of two independent prospective cohort studies; the Nurses' Health Study (N = 121,701 women followed since 1976) and the Health Professionals Follow-up Study (N = 51,529 men followed since 1986).[10] When a participant (or next of kin for decedents) reported colorectal cancer, study physicians reviewed medical records, and recorded TNM stage and tumor location. We collected paraffin-embedded tissue blocks from hospitals where colorectal cancer patients underwent tumor resections.[10] We have previously shown that there is no substantial or significant difference in demographic features or nutritional intakes between cases with and without available tissue.[10] Tissue sections from all available colorectal cancers were reviewed by a pathologist (S.O.). Tumor grade was categorized as high (≤50% glandular area) or low (>50% glandular area). Based on availability of tissue samples and data, we included a total of 470 stage I-IV colorectal cancer cases diagnosed up to 2002 (Table 1). Patients were observed until death or June 2008, whichever came first. Ascertainment of deaths included reporting by the family or postal authorities. In addition, the names of persistent nonresponders were searched in the

National Death Index. The cause of death was assigned by physicians blinded to information on lifestyle exposures and molecular changes in colorectal cancer. Written informed consent was obtained from all study subjects. This study was approved by the Human Subjects Committees at Brigham and Women's Hospital and the Harvard School of Public Health.

Microsatellite Instability (MSI) Analysis and Pyrosequencing of *KRAS, BRAF* and *PIK3CA*

DNA from paraffin-embedded tissue was extracted, and PCR, and pyrosequencing targeted for *KRAS* codons 12 and 13,[11] *BRAF* codon 600,[12] and *PIK3CA* exons 9 and 20 was performed.[13] MSI status was determined using D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67 and D18S487.[14] MSI-high was defined as the presence of instability in ≥30% of the markers, MSI-low as the presence of instability in 1-29% of the markers, and microsatellite stability (MSS) as no unstable marker.

Real-Time PCR for CpG Island Methylation and Pyrosequencing to Measure LINE-1 Methylation

Sodium bisulfite treatment on tumor DNA and subsequent real-time PCR (MethyLight) assays were validated and performed as previously described.[15] We quantified promoter methylation in 8 CIMP-specific genes (*CACNA1G, CDKN2A, CRABP1, IGF2, MLH1, NEUROG1, RUNX3* and *SOCS1*).[16-19] CIMP-high was defined as ≥6/8 methylated promoters using the 8-marker CIMP panel, CIMP-low/0 as 0 to 5 methylated promoters, according to the previously established criteria.[17] In order to accurately quantify relatively high LINE-1 methylation levels, we utilized Pyrosequencing as previously described.[20]

Immunohistochemistry for CDK8 and other proteins

Tissue microarrays (TMAs) including tumor and adjacent normal mucosa were constructed as previously described.[21] Methods of immunohistochemical procedures and interpretation were previously described as follows: CDK8,[4] FASN,[14, 22] β-catenin (*CTNNB1*),[23] cyclin D1 (*CCND1*),[24] p53,[25] p21 (*CDKN1A*), p27 (*CDKN1B*),[25-27] and COX-2 (*PTGS2*).[10, 14] β-catenin score was calculated as the sum of nuclear (0-2), cytoplasmic (0-2) and membrane (0 if there was intact expression; 1 if there was loss of expression) as previously described.[23] β-catenin was interpreted to be active when the β-catenin score was 3-5.[23] CDK8 expression was determined by assessing the level of immunohistochemical staining in tumor cell nuclei (Figures 1 and 2). Initially, the overall staining intensity was scored as none, weak, moderate or strong. Cases categorized as positive were those characterized by weak, moderate or strong staining, while cases categorized as negative were those with no nuclear staining. This cutpoint was based on the relationship of CDK8 staining intensity with β-catenin score and patient survival. Any other method of cutpoint determination would be arbitrary, and not biologically-based. The adjusted colon cancer-specific hazard ratio (HR) compared to CDK8-null cases was: 2.08 (95% CI, 1.14-3.82) for CDK8-weak cases; and 2.05 (95% CI, 1.01-4.18) for CDK8-moderate/strong cases. The frequency of β-catenin activation was: 25% (33/130) in CDK8-null tumors; 39% (74/190) in CDK8-weak tumors; 49% (55/112) in CDK8-moderate/strong tumors. To analyze the expression of CDK8 in normal mucosa, we used normal mucosa adjacent to colorectal cancer for each case. Appropriate positive and negative controls were included in each run of immunohistochemistry. All specimens stained for CDK8 were interpreted independently by two pathologists (R.F. and K.S.) unaware of other data. The concordance between the two observers was 0.82 (κ=0.58, $p<0.0001$), indicating good agreement. Discordant results were resolved by discussion. A random selection of 108-402 cases was examined for each of the other markers by a second observer (p53 and FASN by K.N.; p21 and p27 by K.S.; β-catenin by S.O.; COX-2 by R. Dehari, Kanagawa Cancer Center, Japan) unaware of other data, and the concordance rate between the two observers was always greater than 0.82 [all $\kappa>0.61$ (except for κ=0.57 for FASN), all $p<0.0001$], indicating generally good to substantial agreement.

Statistical Analysis

All statistical analyses were performed using SAS program (Version 9.1, SAS Institute, Cary, NC). All p values were two-sided, and statistical significance was set at $p \leq 0.05$. Nonetheless, when we performed multiple hypothesis testing, p values were conservatively interpreted, and threshold for significance was set at p=0.0024 (=0.05/21). For categorical data, the chi-square test (or Fisher's exact test when any expected cell count was <5). To assess independent relations with CDK8 status, we performed logistic regression analysis, which initially included age at diagnosis (as a continuous variable), sex (female vs. male), body mass index (BMI; <30 vs. ≥30 kg/m^2), family history of colorectal cancer in any first degree relative (present vs. absent), tumor location (right vs. left colon vs. rectum), stage (I-II vs. III-IV), grade (low vs. high), MSI (high vs. low/MSS), CIMP (high vs. low/0), β-catenin score (high, 3-5 vs. low, 0-2), LINE-1 methylation (continuous), *BRAF*, *KRAS*, *PIK3CA* (mutant vs. wild-type), p53, p21, p27, cyclin D1, COX-2 and FASN. We performed a backward stepwise elimination with a threshold of p=0.20, to limit the number of variables in the final model and avoid overfitting. We assigned separate "missing" indicator variables to those cases with missing data in any of the categorical covariates.

For survival analysis, the Kaplan-Meier method was used to describe the distributions of colon cancer-specific and overall survival time, and the log-rank test was performed. For analyses of colorectal cancer-specific mortality, death as a result of colorectal cancer was the primary end point and deaths as a result of other causes were censored. To assess potential confounding, we used stage-matched (stratified) Cox proportional hazards models and calculated hazard ratio (HR) according to CDK8 status. The initial model included age (continuous), sex, year of diagnosis (continuous), BMI, family history of colorectal cancer, tumor location, grade, MSI, CIMP, LINE-1 methylation (continuous), *KRAS*, *BRAF*, *PIK3CA*, p53, p21, p27, cyclin D1, COX-2, β-catenin and FASN. Tumor stage (I, IIA, IIB, IIIA, IIIB, IIIC, IV, unknown) was used as matching (stratifying) variable using the "strata" option in the SAS "proc phreg" command, to avoid residual confounding. We performed a backward stepwise elimination with a threshold of p=0.20, to limit the number of variables in the final model and avoid overfitting. The proportionality of hazards assumption was satisfied by evaluating time-dependent variables, which were the cross-product of the CDK8 variable and survival time (p=0.87 for colon cancer-specific mortality; p=0.86 for overall mortality). Cases with missing data on a given covariate were included in the majority category in that covariate to minimize the number of indicator variables and avoid overfitting. We confirmed that excluding cases with missing data in any of the covariates did not substantially alter results (data not shown). An interaction was assessed by the Wald test on the cross product of CDK8 and another variable of interest in a multivariate Cox model. When assessing an interaction between CDK8 and stage, we dichotomized tumor stage (I-II vs. III-IV) as well as treated stage as a linear ordinal variable (I to IV) to make certain that there was no significant interaction.

Results

CDK8 expression in colorectal cancer

CDK8 resides on a region of chromosome 13 that is known to undergo chromosomal gain in ~60% of colorectal cancers.[4, 28-30] Previous studies have shown that CDK8 is overexpressed in a subset of colorectal cancers,[4, 28, 30] and that its kinase activity is necessary for β-catenin-dependent transcription and for β-catenin-driven cellular transformation.[4, 5] To further understand the significance of CDK8 overexpression, we assessed CDK8 expression in a large cohort of clinically annotated colorectal cancer specimens. Among 470 patients with stage I-IV colorectal cancer, we detected nuclear CDK8 expression in 329 (70%) tumors by immunohistochemistry (Figure 1). We assessed

the frequency of CDK8 expression according to clinical and pathologic features (Table 1). CDK8 expression was more common in women than in men [odds ratio (OR) 1.88; 95% confidence interval (CI) 1.26-2.81; p=0.002]. Age at diagnosis, BMI, tumor location, tumor stage, tumor grade, mucinous component or signet ring cell component was not significantly associated with CDK8 expression.

To better understand the pathogenetic significance of CDK8 expression, we assessed the frequency of CDK8 expression according to molecular characteristics which have been shown to be important in colorectal carcinogenesis (Table 1). Previous studies have implicated CDK8 and the Mediator complex in β-catenin activation in both model organisms[31] and human colon cancer cell lines.[7] Consistent with these studies, we found that tumors expressing CDK8 were associated with high β-catenin activation (OR 2.19; 95% CI, 1.39-3.46; p=0.0006) as determined by an activity score [23] reflecting the combined β-catenin expression in the nucleus and cytoplasm and lack of expression at the membrane (Figure 1 and Table 1). CDK8 expression was also associated with fatty acid synthase (FASN) overexpression (p=0.0003) and p53 expression (p=0.008). FASN has been shown to be overexpressed in a subset of colorectal cancer,[22, 32-34] and associated with microsatellite instability (MSI)[22, 34] and superior survival in non-obese individuals.[35] MSI, CIMP, LINE-1 methylation, *KRAS* mutation, *BRAF* mutation, *PIK3CA* mutation, p21, p27, cyclin D1 or COX-2 expression was not significantly associated with CDK8 expression.

CDK8 expression in cancer and adjacent normal colon mucosa

In 249 cases, we were able to score CDK8 expression in adjacent normal mucosa and compare it with expression in matched colorectal cancer specimens. The majority (131/249; 52%) of “adjacent normal” specimens failed to stain for CDK8 expression. Importantly, 78 (60%) of these 131 cases of CDK8-negative “adjacent normal” cases overexpressed CDK8 in the patient-matched tumor. The majority of “adjacent normal” samples that were positive for CDK8 expression, also showed positivity in the matching tumor samples (102/118; 86%). This correlation might be explained by either cancer field effects or normal population heterogeneity in CDK8 expression.

Multivariate analysis to assess independent relations with CDK8 expression

We performed multivariate logistic regression analysis to examine whether CDK8 expression was independently associated with β-catenin activation or any of the clinical, pathologic and other molecular variables (Table 2). In multivariate analysis, CDK8 expression was independently associated with β-catenin activation (p=0.0002), female gender (p<0.0001), FASN overexpression (p=0.0003), and p53 expression (p=0.004). Of note, a p value of 0.0024 was considered to be a threshold for significance after a correction for multiple testing. These data implied that CDK8 expression was associated with β-catenin activity in human colorectal tumors and suggested additional pathways (such as the energy balance-FASN pathway) associated with CDK8 activity in colorectal cancer.

CDK8 expression and patient mortality in colorectal cancer

To test whether CDK8 expression is associated with patient outcome, we assessed the influence of CDK8 on survival of colorectal cancer patients. Among 452 eligible patients (mean follow-up 8.1 years), there were 202 deaths, including 116 colorectal cancer-specific deaths. Since clinical management for colon cancer patients differs from that for rectal cancer patients, we analyzed the prognostic effect of CDK8 in colon cancer and rectal cancer, separately.

In Kaplan-Meier analysis on 372 patients with colon cancer, 5-year colon cancer-specific survival was significantly lower in CDK8-positive cases than CDK8-negative cases (72%

vs. 82%; log-rank p=0.039) (Figure 2). In univariate Cox regression analysis, CDK8-expressing colon cancer cases were significantly associated with high colon cancer-specific mortality [hazard ratio (HR) 1.70; 95% CI, 1.03-2.83; p=0.039] (Table 3). When we assessed the independent prognostic effect of CDK8 by a multivariate Cox model that adjusted for other predictors of patient survival, CDK8 expression was significantly associated with high colon cancer-specific mortality (multivariate HR 2.05; 95% CI, 1.18-3.56; p=0.011). The greater multivariate HR (2.05) than unadjusted HR (1.70) was principally due to adjusting for β-catenin, FASN and LINE-1 methylation. When we adjusted for these variables, the adjusted HR for colon cancer-specific mortality for CDK8-positive tumors was 2.04 (95% CI, 1.22-3.41; p=0.0064). No other major confounder was found. Though somewhat attenuated, similar results were observed upon the assessment of overall mortality (Table 3).

In contrast to colon cancer patients, among 80 rectal cancer patients, rectal cancer-specific or overall mortality did not significantly differ according to CDK8 status in Kaplan-Meier analysis (log-rank p>0.40), or in univariate or multivariate Cox regression analysis (p>0.30) (Table 3).

Examination of modifying effect on the relation between CDK8 and prognosis

We examined whether the influence of CDK8 expression on colon cancer-specific mortality was modified by any of the clinical, pathologic and other molecular features of the tumors (age, sex, year of diagnosis, BMI, family history of colorectal cancer, tumor location, grade, stage, MSI, CIMP, LINE-1 methylation, *KRAS*, *BRAF*, *PIK3CA*, p53, p21, p27, cyclin D1, COX-2, β-catenin and FASN). We did not identify evidence for significant effect modification by any of the variables (all $P_{interaction}$ >0.20). Notably, the prognostic effect of CDK8 did not significantly differ between the two independent cohort studies ($P_{interaction}$ =0.79), or between low stage (I-II) and high stage (III-IV) cases ($P_{interaction}$ =0.64).

Discussion

We conducted this study to examine CDK8 expression in a large cohort of colorectal cancers which were annotated with clinical, pathologic and molecular information. A copy number gain of 13q where the *CDK8* gene resides has been reported as a frequent event in the transition from colorectal adenoma to carcinoma.[36] CDK8 is commonly expressed in colon cancer and its overexpression highly correlates to copy number gain of its locus (13q12.13).[4] We have found that CDK8 expression is associated with high β-catenin activity as well as other molecular markers of significance in colon cancer such as fatty acid synthase (FASN) and p53 expression. Importantly, CDK8 expression is significantly associated with a poor patient outcome in human colon tumors independent of stage and other potential predictors of patient outcome. In contrast to colon cancer patients, tumoral CDK8 status does not appear to predict outcome among rectal cancer patients. Our findings support a role for CDK8 in human colon cancer and define CDK8 positive tumors as having a unique clinico-pathological footprint.

A role for CDK8 in β-catenin driven transcription has been documented in colon cancer cell lines. [7] Furthermore, in *Drosophila melanogaster*, additional Mediator protein subunits MED12 and MED13, drive β-catenin activity.[31] Given the importance of the Wnt/β-catenin pathway in human tumorigenesis, our data showing an association between CDK8 expression and β-catenin activity are particularly intriguing. While it is possible that the presence of poor quality specimens, which tended to be negative for any markers, might have driven the relationship between CDK8 and β-catenin nuclear and cytoplasmic expressions towards a concordant pattern, the association between CDK8 and loss of β-catenin membrane expression was reassuring for a true relationship. Our finding raises the

possibility that CDK8 may affect β-catenin protein stability or nuclear retention/localization. Further studies will be of interest to determine whether the association between CDK8 expression and β-catenin activity is correlative or causative. These results support a role for CDK8 in β-catenin driven human malignancies and lend credence to the possibility of CDK8 being a therapeutic target for β-catenin driven cancers.

Over the past decade, cyclin-dependent kinases (CDKs), have been shown to be hyperactivated in multiple tumor types.[37] As such, there are a number of CDK inhibitors currently being developed and undergoing clinical testing in several human malignancies, including colon cancer.[37] Previous studies have shown that CDK8 kinase activity is critical for regulating β-catenin activity in colon cancer.[4, 5] Therefore, it will be of interest to test such small molecules for their ability to inhibit CDK8. Our results identify a patient population that is characterized by high levels of CDK8 activity, providing a potential patient pool that may be distinctly responsive to CDK inhibitor therapy. Future studies focusing on the ability of known CDK inhibitors to target CDK8 will be particularly useful in assessing whether CDK8 expression can serve as a therapeutic biomarker for clinical trials involving CDK inhibitory molecules.

Examining molecular biomarkers is important in cancer research.[38-46] Our data suggest that CDK8 expression may be associated with a poor patient prognosis, independently of potential confounders, including β-catenin. These results are consistent with the fact that CDK8 is involved in regulating other pathways besides Wnt/β-catenin and suggest that CDK8 inhibition may also be useful in tumors which may not be driven by β-catenin hyperactivity. Nonetheless, our findings need to be confirmed by independent studies.

In addition to the potential association with β-catenin activity and patient outcome, we have found that CDK8 expression is potentially associated with two other important molecules related to cancer; p53 and FASN (fatty acid synthase).[22, 32-35] The potential association between CDK8 and p53 is particularly intriguing, as a previous study has suggested that increased binding of CDK8 to p53 target genes correlates positively with transcriptional strength.[9] Given the important role of *TP53* mutation in multiple human tumors, it will be of great interest to further study the association between CDK8 and *TP53* mutation in driving human malignancies. The relationship between CDK8 expression and FASN may be more indirect given that the two proteins do not share a common intracellular space. FASN is activated in states of energy excess and overexpressed in multiple human malignancies, including colorectal cancer.[22, 32-35] Although no studies have directly linked CDK8 to energy balance, our data are consistent with the pleiotropic roles of CDKs in cellular homeostasis and may open new avenues of study in CDK8 and energy metabolism. These associations between CDK8 and β-catenin activity, p53 expression and FASN expression define a set of molecular features of CDK8-positive tumors characterized by increased oncogenic activity.

We found an intriguing association between female gender and CDK8 expression. Female gender has been known to be associated with some molecular events such as CpG island methylation. In particular, the CpG island methylator phenotype (CIMP) has been associated with female gender.[19, 47] Postmenopausal hormone use has been known to modify the risk of developing colorectal cancer,[48] as well as risk of colorectal cancer mortality.[49, 50] Therefore, it is conceivable that a different hormonal environment between male and female may predispose to different molecular aberrations, including CDK8 amplification and overexpression. Further studies are necessary for understanding of biologic basis of the association between female gender and CDK8 expression in colorectal cancer.

There are limitations in this study. For example, data on cancer treatment were limited. Nonetheless, it is unlikely that chemotherapy use substantially differed according to CDK8 status, since such data were unavailable for clinicians. In addition, beyond cause of mortality, data on cancer recurrences were not available in these cohorts. Nonetheless, given the median survival for metastatic colon cancer was approximately 10 to 12 months during much of the time period of this study, colorectal cancer-specific survival should be a reasonable surrogate for cancer-specific outcomes.

There are advantages in utilizing the database of the two independent prospective cohort studies, the Nurses' Health Study and Health Professionals Follow-up Study to examine significance of tumoral CDK8 expression. Anthropometric measurements, family history of cancer, other clinical information, pathologic and tumor staging data, and tumoral molecular features were prospectively collected, and entered into the database blinded to patient outcome. Cohort participants who developed colon cancer were treated at hospitals throughout the United States, and thus more representative of colorectal cancers in the general population, than studies based on a single to few hospitals. Tumor specimen procurement rate has been 60-70%, and there were no demographic difference between cases with tumor tissue analyzed and those without tumor tissue analyzed.[10] In addition, our rich tumor database enabled us to simultaneously assess pathologic and molecular features of tumor and control for confounding by tumoral variables. Thus, our study is unique in terms of comprehensiveness of tumor database.

In summary, we have conducted the first population-based study to assess CDK8 expression in a large cohort of colorectal cancers. Our findings show that CDK8 is expressed in a high fraction of colorectal cancers and that CDK8-positive colon cancers are significantly associated with a poor patient prognosis. These data define a molecular and clinical landscape for CDK8-positive colon cancers. Given the number of CDK inhibitors undergoing clinical trials for a variety of human malignancies, these findings may be of great use in defining patients that may be distinctly susceptible to CDK small molecule based therapies.

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Abbreviations and the HUGO Gene Nomenclature Committee-approved official gene symbols

BMI	body mass index
CDK8	cyclin-dependent kinase 8
CI	confidence interval
CIMP	CpG island methylator phenotype

COX-2	cyclooxygenase-2
FASN	fatty acid synthase
HR	hazard ratio
MSI	microsatellite instability
MSS	microsatellite stable
OR	odds ratio

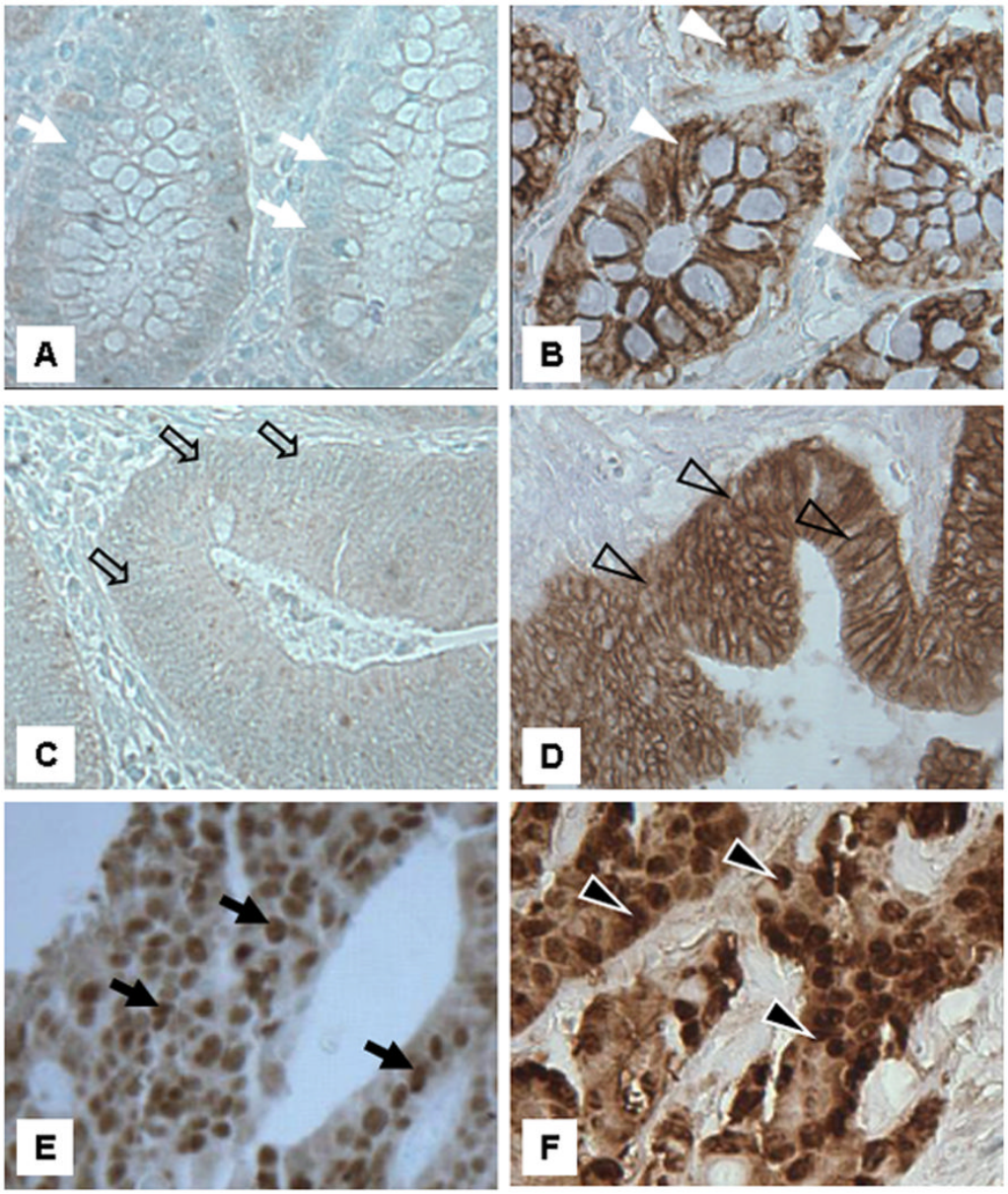
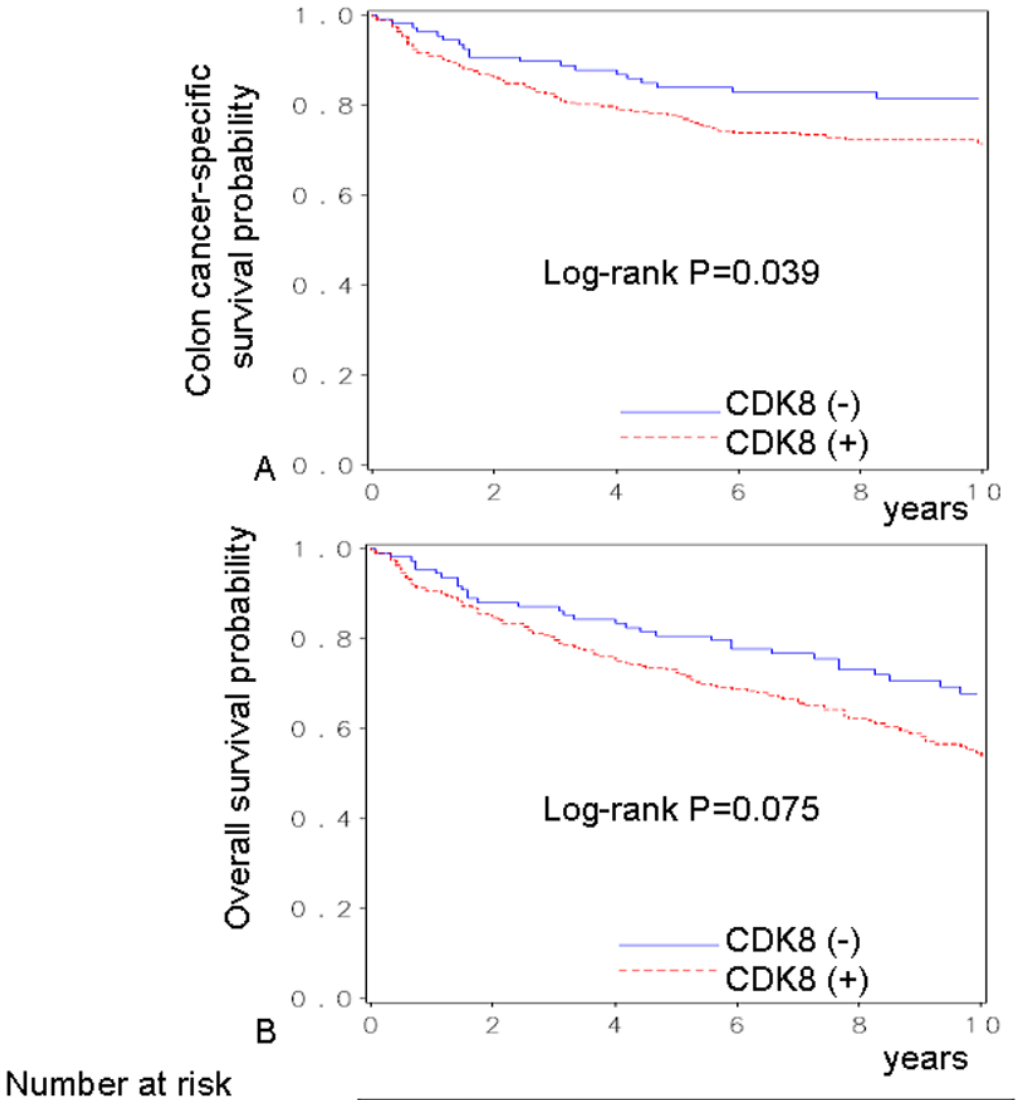


Figure 1.

CDK8 (left panel) and β-catenin (right panel) expression in colon cancer and adjacent normal mucosa.

CDK8 overexpression is absent (A, white arrows) and β-catenin expression is membranous (B, white arrowheads) in normal mucosa adjacent to colon cancer. CDK8 overexpression is absent (C, arrows) and β-catenin expression is membranous (D, arrowheads) in colon cancer. Colon cancer cells show nuclear CDK8 overexpression (E, black arrows) and nuclear β-catenin expression with loss of membrane β-catenin expression (F, black arrowheads).



Number at risk

Year	0	2	4	6	8	10
CDK8 (-)	108	95	90	79	59	41
CDK8 (+)	264	224	197	166	119	86

Figure 2.

Kaplan-Meier survival curves according to CDK8 status in colon cancer. A. Colon cancer-specific survival. B. Overall survival. The table shows the number of patients who were alive at each time point after the diagnosis of colon cancer.

Table 1

Frequency of CDK8 expression in colorectal cancer according to clinical, pathologic or molecular feature.

Clinical, pathologic or molecular feature	Total N	CDK8+	Univariate OR (95% CI)	P value
All cases	470	329 (70%)		
Gender				
Men	183	113 (62%)	1	Referent
Women	287	216 (75%)	1.88 (1.26-2.81)	0.002
Age				
<65	202	136 (41%)	1	
≥65	268	193 (59%)	1.25 (0.84-1.86)	
Body mass index (BMI, kg/m^2)				
<30	375	263 (70%)	1	
≥30	80	57 (71%)	1.06 (0.62-1.80)	
Tumor location				
Right colon (cecum to transverse colon)	229	166 (72%)	1	
Left colon (splenic flexure to sigmoid)	155	108 (70%)	0.87 (0.56-1.37)	
Rectum	81	51 (63%)	0.64 (0.38-1.10)	
Tumor stage				
I	102	74 (73%)	1	
II	148	103 (70%)	0.87 (0.50-1.51)	
III	128	87 (68%)	0.80 (0.45-1.42)	
IV	66	47 (71%)	0.94 (0.47-1.86)	
Unknown	26	18 (69%)	0.85 (0.33-2.18)	
Tumor grade				
Low	415	288 (69%)	1	
High	42	30 (71%)	1.10 (0.55-2.22)	
Mucinous component				
Absent	260	178 (68%)	1	
Present	153	117 (76%)	1.50 (0.95-2.36)	
Signet ring cell component				
Absent	369	259 (70%)	1	
Present	24	18 (75%)	1.27 (0.49-3.30)	
MSI status				
MSS	338	237 (70%)	1	
MSI-low	50	34 (68%)	0.91 (0.48-1.71)	
MSI-high	81	58 (72%)	1.07 (0.63-1.84)	
CIMP status (No. of methylated CIMP markers)				
CIMP-0 (0)	203	140 (69%)	1	
CIMP-low (1-5)	187	129 (69%)	1.00 (0.65-1.54)	
CIMP-high (6-8)	69	50 (72%)	1.18 (0.65-2.17)	
LINE-1 methylation				
≥60%	247	172 (70%)	1	

Clinical, pathologic or molecular feature	Total N	CDK8+	Univariate OR (95% CI)	P value
<60%	206	142 (69%)	0.97 (0.65-1.44)	
BRAF mutation				
(-)	398	274 (69%)	1	
(+)	62	49 (79%)	1.71 (0.89-3.26)	
KRAS mutation				
(-)	301	213 (71%)	1	
(+)	168	115 (68%)	0.90 (0.60-1.35)	
PIK3CA mutation				
(-)	357	248 (69%)	1	
(+)	60	39 (65%)	0.82 (0.46-1.45)	
FASN (fatty acid synthase) expression				
(-)	327	220 (67%)	1	Referent
(+)	60	54 (90%)	4.38 (1.83-10.5)	0.0004
p53 expression				
(-)	280	183 (65%)	1	Referent
(+)	186	143 (77%)	1.76 (1.16-2.68)	0.008
p21 (*CDKN1A*)				
Expressed	93	64 (69%)	1	
Lost	367	255 (69%)	1.03 (0.63-1.69)	
p27 (*CDKN1B*)				
Nuclear expression	96	72 (75%)	1	
Cytoplasmic expression or loss of expression	353	244 (69%)	0.75 (0.45-1.25)	
cyclin D1 expression				
(-)	126	89 (71%)	1	
(+)	323	228 (71%)	1.00 (0.63-1.57)	
COX-2 (cyclooxygenase-2) expression				
(-)	81	50 (62%)	1	
(+)	389	279 (72%)	1.57 (0.95-2.59)	
β-catenin, nuclear				
Negative (0)	193	124 (64%)	1	Referent
1+	136	100 (74%)	1.55 (0.96-2.50)	
2+	103	78 (76%)	1.74 (1.01-2.97)	0.043
β-catenin, cytoplasmic				
Negative (0)	250	156 (62%)	1	Referent
1+	152	122 (80%)	2.45 (1.52-3.94)	0.0002
2+	30	24 (80%)	2.41 (0.95-6.11)	
β-catenin, membrane				
Expressed (0)	195	127 (65%)	1	Referent
Lost (1+)	237	175 (74%)	1.51 (1.00-2.28)	0.049
β-catenin, overall score*				
0-2 (inactive)	270	173 (64%)	1	Referent
3-5 (active)	162	129 (80%)	2.19 (1.39-3.46)	0.0006

Clinical, pathologic or molecular feature	Total N	CDK8+	Univariate OR (95% CI)	P value
CDK8 in adjacent normal colon				
(-)	131	78 (60%)	1	Referent
Weak (+)	82	68 (83%)	3.30 (1.68-6.47)	0.0003
Moderate (+)	36	34 (94%)	11.6 (2.66-50.1)	<0.0001

Only significant p values are described.

*β-catenin score was calculated as the sum of nuclear (0-2), cytoplasmic (0-2) and membrane (0-1) scores as previously described.[23]

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

Table 2

Multivariate analysis of the relations with CDK8 expression in colorectal cancer

Variables in multivariate logistic regression model including CDK8 as outcome variable	Multivariate OR (95% CI)	P value
Female	2.66 (1.68-4.23)	<0.0001
β-catenin (overall score 3-5 vs. 0-2)*	2.61 (1.58-4.29)	0.0002
FASN (fatty acid synthase) expression	5.36 (2.17-13.3)	0.0003
p53 expression	1.99 (1.25-3.17)	0.004
Rectal location (vs. proximal location)	0.45 (0.24-0.83)	0.010
Age (10-year increment as a unit)	1.42 (1.08-1.87)	0.011

Multivariate logistic regression analysis initially included in all of the variables. Backward stepwise elimination with a threshold of p=0.20 excluded body mass index, family history of colorectal cancer, tumor stage, grade, MSI, CIMP, p21, p27, cyclin D1, COX-2, LINE-1 methylation, *BRAF*, *KRAS* and *PIK3CA*. The remaining variables are listed in the table. A p value of 0.0024 was considered as a threshold for significance after a correction for multiple testing.

*β-catenin score was calculated as the sum of nuclear (0-2), cytoplasmic (0-2) and membrane (0-1) scores as previously described.[23]

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

Table 3

CDK8 expression in colorectal cancer and patient mortality

	Total N	Colorectal cancer-specific mortality				Overall mortality			
		Deaths / person-years	Univariate HR (95% CI)	HR (95% CI) adjusted for LINE-1, FASN and β-catenin*	Multivariate HR (95% CI)	Deaths / person-years	Univariate HR (95% CI)	HR (95% CI) adjusted for LINE-1, FASN and β-catenin*	Multivariate HR (95% CI)
Colon cancer									
CDK8 (-)	108	19/984	1 (referent)	1 (referent)	1 (referent)	42/984	1 (referent)	1 (referent)	1 (referent)
CDK8 (+)	264	73/2066	1.70 (1.03-2.83)	2.04 (1.22-3.41)	2.05 (1.18-3.56)	124/2066	1.38 (0.97-1.96)	1.53 (1.07-2.19)	1.46 (1.00-2.14)
P value			0.039	0.0064	0.011		0.08	0.021	0.051
Rectal cancer									
CDK8 (-)	29	11/227	1 (referent)	1 (referent)	1 (referent)	16/227	1 (referent)	1 (referent)	1 (referent)
CDK8 (+)	51	13/383	0.70 (0.31-1.57)	0.87 (0.39-1.97)	0.95 (0.40-2.25)	20/383	0.75 (0.39-1.45)	0.85 (0.44-1.66)	0.80 (0.40-1.59)
P value			0.39	0.74	0.91		0.39	0.63	0.52

The multivariate, stage-matched (stratified) Cox regression model initially included the CDK8 variable stratified by location (colon vs. rectum) and all other covariates. Backward stepwise elimination excluded sex, body mass index, family history of colorectal cancer, *KRAS*, *BRAF*, *PIK3CA*, CIMP, cyclin D1, p53 and p21. Age, year of diagnosis, grade, p27, COX-2, fatty acid synthase (FASN), β-catenin, LINE-1 methylation and MSI remained in the model. CI, confidence interval; HR, hazard ratio.

*No other major confounders were present.