SIRT1 Histone Deacetylase Expression Is Associated with Microsatellite Instability and CpG Island Methylator Phenotype in Colorectal Cancer

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Abstract

The class III histone deacetylase SIRT1 plays an important role in epigenetic gene silencing. Inhibition of SIRT1 reactivates silenced genes, suggesting a possible therapeutic approach of targeted reversal of aberrantly silenced genes. In addition, SIRT1 may play a role in the well-known link between obesity, cellular energy balance and cancer. However, a comprehensive study of SIRT1 using human cancer tissue with clinical outcome data is currently lacking, and its prognostic significance is uncertain. Utilizing the database of 485 colorectal cancers in two independent prospective cohort studies, we detected SIRT1 overexpression in 180 (37%) tumors by immunohistochemistry. We examined its relationship to the CpG island methylator phenotype, related molecular events, clinical features including body mass index, and patient survival. We quantified DNA methylation in 8 CIMP-specific promoters (*CACNA1G, CDKN2A, CRABP1, IGF2, MLH1, NEUROG1, RUNX3, SOCS1*) and 8 other CpG islands (*CHFR, HIC1, IGFBP3, MGMT*, MINT1, MINT31, p14, *WRN*) by MethyLight. SIRT1 overexpression was associated with CIMP-high (6/8 methylated CIMP-specific promoters, p=0.002) and MSI-high, (p<0.0001). In both univariate and multivariate analyses, SIRT1 overexpression was significantly associated with the CIMP-high MSI-high phenotype (multivariate odds ratio, 3.20; 95% confidence interval, 1.35–7.59; p=0.008). In addition, mucinous component (p=0.01), high tumor grade (p=0.02) and fatty acid synthase overexpression (p=0.04) were significantly associated with SIRT-positivity in

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multivariate analysis. SIRT1 was not significantly related with age, sex, tumor location, stage, signet ring cells, cyclooxygenase-2, LINE-1 hypomethylation, *KRAS, BRAF, BMI, PIK3CA, HDAC*, p53, β-catenin, COX-2, or patient prognosis. In conclusion, SIRT1 expression is associated with CIMP-high MSI-high colon cancer, suggesting a role of SIRT1 in gene silencing in this unique tumor subtype.

Keywords

colon cancer; epigenetics; SIRT; HDAC; DNA methylation; histone; acetylation

INTRODUCTION

Various histone modifications that affect chromatin structures represent an important epigenetic mechanism of gene silencing.[1, 2] DNA methylation and histone modifications seem to form reinforcing networks for stable gene silencing during carcinogenic process.[1, 2] SIRT1, which is one of the class III histone deacetylases,[1] is a human homolog of the SIR2; a protein that is activated during calorie restriction and has been associated with increased lifespan.[3–6] The role of SIRT1 in cancer is controversial, and perhaps multifaceted.[7–17] On one hand, its ability to deacetylate p53 implies its role as an oncogene,[11–13] while other evidence suggests its tumor suppressor activity by deacetylating β-catenin.[14, 17] In colon cancer cells, inhibition of SIRT1 reactivates silenced genes even with retention of DNA methylation.[16] These data collectively imply a link between DNA methylation, SIRT1 and cancer, and suggest the possibility of targeted reversal of aberrantly silenced tumor suppressor genes. In addition, SIRT1 may play a role in the well-known link between obesity, cellular energy balance and cancer. However, a large-scale study of SIRT1 expression using human cancer tissue is currently lacking.

The CpG island methylator phenotype (CIMP) is a major epigenetic phenotype in colorectal cancer, and characterized by widespread CpG island methylation.[18–21] CIMP-high in colorectal cancer has been associated with older age, female, proximal tumor location, *BRAF* mutation, microsatellite instability (MSI), wild-type *TP53* and stable chromosomes.[22–26] Although overexpression of SIRT1 has been reported in human colorectal cancer,[17, 27] the relationship between SIRT1 and CIMP is uncertain.

In this study, we assessed SIRT1 expression in 485 colorectal cancers, and examined its relationship to CIMP, related molecular events, clinical features (including obesity) and prognosis. We have found that SIRT1 expression is associated with CIMP and MSI, independent of other clinical and molecular variables.

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),[28, 29] and the Health Professional Follow-up Study (N = 51,500 men followed since 1986).[29] Data on height and weight were obtained by biennial questionnaire. A subset of the cohort participants developed colorectal cancers

during prospective follow-up. 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.[28, 29] Data on tumor location and stage were obtained through medical record review. 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 and results, a total of 485 colorectal cancers were included. Written informed consent was obtained from all study subjects. Among our cohort studies, there was no significant difference in demographic features between cases with tissue available and those without available tissue.[29] This current analysis represents a new analysis of SIRT1 in the well-established colorectal cancer database,[29–32] which is analogous to novel studies using the well-described cell lines or animal models. In any of our previous studies, we have not examined SIRT1 expression or the relations of SIRT1 with clinical, outcome or other molecular variables. This study represents a unique novel study in term of 1) a large sample size analyzed for SIRT1; 2) the clinical and tissue molecular database, including the long-term follow-up outcome data; and 3) a number of molecular variables that have been analyzed. Tissue collection and analyses were approved by the Harvard School of Public Health 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.) unaware of other data. The tumor grade was categorized as low (50% gland formation) vs. high (<50% gland formation). The presence and extent of extracellular mucin were categorized as 0% (no mucin), 1–49% or 50% of the tumor volume. The presence and extent of signet ring cells were categorized as 0% (no signet ring cells) or 1% of the tumor volume.

Sequencing of *KRAS, BRAF* and *PIK3CA*, and microsatellite instability (MSI) analysis

DNA was extracted from dissected tumor tissue sections, and PCR and Pyrosequencing targeted for *KRAS* (codons 12 and 13),[33] *BRAF* (codon 600)[34] and *PIK3CA* (exons 9 and 20)[35] were performed as previously described. MSI analysis was performed, using 10 microsatellite markers (D2S123, D5S346, D17S250, BAT25, BAT26, BAT40, D18S55, D18S56, D18S67 and D18S487).[36] MSI-high was defined as the presence of instability in 30% of the markers. MSI-low was defined as instability in <30% of the markers, and "microsatellite stable (MSS)" tumors were defined as tumors without an unstable marker.

Real-time PCR (MethyLight) to measure CpG island methylation

Sodium bisulfite treatment on genomic DNA and subsequent real-time PCR (MethyLight)[37] were validated and performed as previously described.[38] We quantified DNA methylation in 8 CIMP-specific promoters [*CACNA1G, CDKN2A* (p16), *CRABP1, IGF2, MLH1, NEUROG1, RUNX3* and *SOCS1*],[24, 30, 36] all of which were selected from screening of 195 CpG islands.[24, 36] CIMP-high was defined as the presence of 6 of 8 methylated promoters, 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.30 In addition, we quantified DNA methylation in 8 other CpG islands (not in the CIMP panel), including *CHFR, HIC1, IGFBP3, MGMT*, MINT1, MINT31, p14, and *WRN*.39 Primers and probes were previously described.32, 39 The PCR condition for all markers 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.

Pyrosequencing to measure LINE-1 methylation

In order to accurately quantify relatively high methylation levels in LINE-1 repetitive elements, we utilized Pyrosequencing as previously described.40 LINE-1 methylation level measured by Pyrosequencing has been shown to correlate with overall 5-methylcytosine level (i.e., genome-wide DNA methylation level) in tumor cells.41

Immunohistochemistry for p53, β-catenin, COX2, FASN and SIRT1

Tissue microarrays (TMAs) were constructed as previously described.29 Methods of immunohistochemical procedures and interpretations were previously described for p53,42 FASN,43, 44 and β-catenin,45 and COX-2.29, 44 For SIRT1 immunohistochemistry (Figure 1), antigen retrieval was performed, and deparaffinized tissue sections in Antigen Retrieval Citra Solution (Biogenex Laboratories, San Ramon, CA) were treated with microwave for 15 min. Tissue sections were incubated with 3% H_2O_2 (10 min) to block endogenous peroxidase (Dako Cytomation Carpinteria, CA), with 10% normal goat serum (Vector Laboratories, Burlingame, CA) in phosphate-buffered saline (10 min), and with serum-free protein block (Dako Cytomation) (10 min). Primary antibody against SIRT1 (Rabbit monoclonal to SIRT1, 1:100 dilution; Epitomics, Burlingame, CA) was applied, and the slides were maintained overnight at room temperature. Next, we applied anti- rabbit IgG antibody (Biogenex Laboratories) for 20 min, followed by a streptavidin-HRP conjugate (Biogenex Laboratories) for 20 min, diaminobenzidine (5 min) and methyl-green counterstain. Nuclear SIRT1 expression was recorded as no expression, weak expression, or moderate/strong expression. SIRT1 positivity (i.e., overexpression) was defined as the presence of at least focal moderate/strong staining. Appropriate positive and negative controls were included in each run of immunohistochemistry. All immunohistochemically stained slides were interpreted by one of the investigators (SIRT1 and β-catenin by K.N.; p53, COX-2 and FASN by S.O.) unaware of other data. A random selection of 174 cases was examined for SIRT1 by a second observer (K.S.) unaware of other data, and concordance between the two observers was 0.85 (κ=0.68, p<0.0001), indicating substantial agreement. For the other markers, a random selection of 108–402 cases was reexamined for each marker by a second pathologist (p53 and FASN by K.N.; β-catenin by S.O.; COX-2 by R. Dehari, Kanagawa Cancer Center, Japan) unaware of other data, and concordance rates and κ coefficients between the two pathologists were as follows: 0.87 (κ=0.75; N=118) for p53; 0.93 (κ=0.57; N=246) for FASN; 0.83 (κ=0.65; N=402); and 0.92 (κ=0.62; N=108) for COX-2, indicating generally substantial agreement.

Statistical analysis

For categorical data, the chi-square test (or Fisher's exact test when any expected cell count was <5) was performed and odds ratio with 95% confidence interval (CI) was computed. The κ coefficient was calculated to assess an agreement between the two interpreters in immunohistochemistry. To confirm independent relations between SIRT1 and clinical and molecular features, a multivariate logistic regression analysis was performed. Odds ratio was adjusted for age (<65 vs. 65 year old), sex, tumor location (proximal vs. distal), body mass index (30 vs. <30 kg/m^2), tumor stage (I–II vs. III–IV), grade (low vs. high), mucin (present vs. absent), signet ring cells (present vs. absent), CIMP/MSI status (CIMP-high MSI-high vs. all other CIMP/MSI subtypes), LINE-1 methylation (as a continuous variable), p53, β-catenin, FASN, COX2, *BRAF, KRAS* and *PIK3CA*. We also examined the possibility of non-linear relations between age and SIRT1 and between body mass index and SIRT1, non-parametrically with restricted cubic splines.46 This method allowed us to examine the relations with SIRT1 without any categorization of age or body mass index.

For survival analysis, the Kaplan-Meier method was used to compare survival time distributions between SIRT1-positive and SIRT1-negative patients. Multivariate, stage-matched conditional Cox proportional hazard models computed hazard ratios (HRs) according to SIRT1 status, adjusted for age, sex, year of diagnosis, tumor location, stage, grade, CIMP, MSI, *KRAS, BRAF, PIK3CA*, p53, β-catenin, FASN, COX-2 and LINE-1 methylation. We also performed univariate Cox regression to assess main effect of SIRT1 on patient mortality. An interaction was assessed by including the cross product of the SIRT1 variable and another variable of interest in a multivariate Cox model, and the likelihood ratio test was performed. All statistical analyses used SAS program (Version 9.1, SAS Institute, Cary, NC). All p values were two-sided, and statistical significance was set at p 0.05; however, p values were conservatively interpreted, considering multiple hypotheses testing.

RESULTS

SIRT1 expression in colorectal cancers

Among the 485 colorectal cancers assessed by immunohistochemistry, 180 (37%) tumors showed nuclear overexpression of SIRT1 (Figure 1). Table 1 summarizes the frequencies of SIRT1 overexpression in relation to various clinical and pathologic features. SIRT1 overexpression was significantly associated with high tumor grade (p=0.003) and mucinous component (50% mucin, p=0.04). Because of the potential links between SIRT1 and aging, 6, 9 and between SIRT1, calorie restriction and cellular energy balance,4, 6 we examined the relations between SIRT1 expression and patient age, and between SIRT1 expression and body mass index (body mass index), non-parametrically with restricted cubic splines46 (Figure 2). This method allowed us to examine the relations to SIRT1 without any categorization of age or body mass index. However, there was no significant association of SIRT1 expression with patient age or body mass index.

SIRT1 overexpression is associated with MSI-high and CIMP-high

Table 2 summarizes the frequencies of SIRT1 overexpression in relation to molecular alterations in colorectal cancer. SIRT1 overexpression was significantly more common in microsatellite instability-high (MSI-high) tumors (59%=49/83, p<0.0001) than in microsatellite stable (MSS) tumors (34%=117/345). 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 *SOCS1*).24, 30, 36 SIRT1 overexpression was significantly more common in CIMP-high tumors (57%=42/74, p=0.002) than in CIMP-0 tumors (36%=76/209).

In order to examine combined effect of MSI and CIMP on SIRT1 expression, we classified tumors into 4 subtypes according to MSI and CIMP status (Table 2). SIRT1 overexpression was more common in CIMP-high MSI-high tumors (67%=35/52) than all other subtypes (32–46%).

SIRT1 and other molecular changes

SIRT1 expression was not significantly associated with LINE-1 methylation, or alteration in *KRAS, BRAF, PIK3CA*, p53, β-catenin, or COX-2 (Table 2). SIRT1 expression was associated with FASN overexpression (p=0.008).

Relations between SIRT1 and methylation in individual CpG islands

Since SIRT1 expression is associated with CIMP-high, we examined whether SIRT1 expression was related with methylation in any specific individual CpG island. We examined the 8 CIMP-panel markers (*CACNA1G, CDKN2A, CRABP1, IGF2, MLH1, NEUROG1, RUNX3* and *SOCS1*) as well as 8 other CpG islands (*CHFR, HIC1, IGFBP3, MGMT*, MINT1, MINT31, p14 and *WRN*). SIRT1 expression was significantly associated with hypermethylation at *CACNA1G, IGF2, MLH1, NEUROG1, RUNX3, SOCS1*, MINT31 and p14 (Supplemental Table).

The association between SIRT1 overexpression and CIMP-high MSI-high tumors according to *BRAF* status

Because *BRAF* mutation has been tightly linked to CIMP-high, we examined the frequency of the CIMP-high MSI-high phenotype according to SIRT1 and *BRAF* status (Figure 3). Among *BRAF*-mutated tumors, SIRT1 expression was significantly associated with the CIMP-high MSI-high phenotype [odds ratio (OR) 10.4; 95% CI, 3.39–32.0; p<0.0001]. Notably, the frequency of the CIMP-high MSI-high phenotype was 74% (23/31) in *BRAF*-mutated SIRT1-positive tumors in contrast to only 6.3% (28/441) in all other subtypes combined (i.e., *BRAF*-wild-type or SIRT1-negative tumors).

SIRT1 is independently associated with CIMP-high MSI-high subtype

We performed multivariate logistic regression analysis, to confirm that the relation between SIRT1 and MSI-high CIMP-high subtype was independent of any other clinical and molecular variables (Table 3). SIRT1 was associated with CIMP-high MSI-high (multivariate OR, 3.20; 95% CI, 1.35–7.59; p=0.008) independent of any other variables.

Mucinous component, high tumor grade and FASN expression were also independently associated with SIRT1. However, significance levels were lower (p values between 0.01 and 0.05) and any of these associations might be a chance event given multiple hypothesis testing.

SIRT1 expression and patient survival

We assessed the influence of SIRT1 overexpression on survival of patients with stage I–IV colorectal cancers. In Kaplan-Meier analysis, SIRT1 expression was not related with colorectal cancer-specific (log rank p=0.63) or overall survival (log rank p =0.87). We performed Cox regression analysis to assess mortalities according to SIRT1 status (Table 4). For both cancer-specific and overall mortalities, SIRT1 was not significantly related with patient mortality in univariate analysis, stage-matched analysis, or multivariate analysis. When we limited cases to only colon cancers, SIRT1 remained unrelated with patient outcome, despite the fact that we have previously shown that molecular features in colon cancer such as CIMP, *BRAF* mutation and LINE-1 methylation are highly associated with prognosis in our cohort studies.[31, 32]

We examined whether SIRT1 was associated with patient mortality in any of the strata of clinical or molecular variables (such as age, sex, tumor stage, location, CIMP, MSI, *BRAF*, LINE-1, etc.). However, there was no evidence for significant interaction between SIRT1 and any of the variables in survival analysis (data not shown).

DISCUSSION

We conducted this study to examine the relations of the class III histone deacetylase (HDAC) SIRT1 with the CpG island methylator phenotype (CIMP), related molecular events and patient outcome in colorectal cancer. Molecular correlates with SIRT1 activation may be important for better understanding of epigenetic and epigenomic aberrations during the carcinogenic process. We have found that SIRT1 expression is significantly associated with CIMP-high and microsatellite instability (MSI). Moreover, SIRT1 expression is significantly associated with the CIMP-high MSI-high phenotype, independent of other clinical and molecular variables. In contrast, SIRT1 expression is not related with global DNA methylation level as measured in LINE-1 repetitive sequence. Our data support the hypothesis that SIRT1 is related with methylation at individual CpG islands, but not with global DNA methylation, in colorectal cancer.

Studying molecular changes is important in cancer research.[47–53] To measure DNA methylation, we utilized real-time PCR (MethyLight technology) for DNA methylation at the 8 CIMP-specific loci [30] and 8 other CpG islands. We also utilized Pyrosequencing to measure LINE-1 methylation that has been correlated with cellular 5-methylcytosine level (i.e., genome-wide DNA methylation level).[41] 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 SIRT1 overexpression, CIMP-high, MSI-high, etc.). The large number of cases has also provided a sufficient power in our multivariate logistic regression analysis and survival analysis.

Recent studies have reported that up-regulation of SIRT1 may prolong cell survival through multiple mechanisms, and play an important role in the regulation of epigenetic alterations. 1, 2, 16, 17 In addition, SIRT1 silences genes through deacetylation of the histone residue, H4K16.8, 54, 55 Our data are likely important, because no study has demonstrated the relationship between SIRT1 and CIMP in human colorectal cancer. On the other hand, our data do not support a direct link between SIRT1 and genome-wide DNA methylation level. SIRT1 has been reported to localize to the promoters of several aberrantly silenced tumor suppressor genes in colon cancer cells, in which CpG islands are hypermethylated, but not to these same promoters in cell lines in which the promoters are not hypermethylated and the genes are expressed.16 These experimental data are consistent with our data of the positive association between SIRT1 and CIMP-high, but no significant relation between SIRT1 and genome-wide DNA methylation level.

With regard to the relationship between MSI and HDACs, a recent study has reported the presence of a truncating mutation in HDAC2 (class I) in MSI-high colorectal cancers.56 However, no study has reported the relation between SIRT1 and MSI. It is important to analyze both CIMP and MSI to decipher the interrelationship between SIRT1, CIMP and MSI. In the current study, we have shown the significant association between SIRT1 and the CIMP-high MSI-high subtype, and it is particularly strong among *BRAF*-mutated cancers. Further studies are necessary to elucidate the relation between SIRT1 activity, *BRAF*, MSI and CIMP.

Recent studies have reported that epigenetic inactivation of *HIC1* results in up-regulation of SIRT1, which deacetylates p53, and that SIRT1 down-regulates p53 through histone deacetylation.15, 16 In addition, SIRT1 has been reported to down-regulate β-catenin through deacetylation and suppresses its ability to facilitate transcription and cell proliferation.17 However, we failed to show associations of SIRT1 with *HIC1* methylation, p53 expression and β-catenin activation. Possible explanations include a difference in patient cohorts, and false positive/negative results in immunohistochemistry. In particular, the presence of poorly preserved tissue specimens might show false negative results on either SIRT1 or β-catenin, which might obscure the inverse relation between nuclear β-catenin and SIRT1 expression. Nonetheless, our classification of SIRT1 status appeared to be valid, since we were able to show the strong association between SIRT1 and the CIMP-high MSI-high subtype.

SIRT1 has been reported to be induced by calorie restriction in multiple tissues of mammals. 3–5 Moreover, at the cellular level, SIRT1 may facilitate this process by regulating energy metabolism.8 Although we have shown no significant relation between patient body mass index and SIRT1 expression, we have shown the relation between SIRT1 and FASN. These results suggest that SIRT1 may cooperate with FASN in regulating energy metabolism in cancer cells.

Many studies have reported anti-tumor effects of HDAC inhibitors, DNA methyltransferase inhibitors and histone lysine demethylases.1, 2, 57, 58 Interestingly, a recent study has reported that blocking SIRT1 function synergizes with both promoter demethylation and inhibition of class I and II HDACs for gene reactivation.16 Moreover, this inhibition of

SIRT1 leads to gene reactivation even with retention of DNA methylation.16 These results suggest new directions for targeting reversal of abnormal gene silencing and demonstrate the importance of ongoing and future studies, which may lead to the eventual translation into clinical practice. In the current study, we have demonstrated a significant association between SIRT1 and CIMP-high MSI-high colorectal cancer. These findings may indicate that therapies targeting SIRT1 may be particularly useful for this CIMP-high MSI-high subtype of cancer.

In conclusion, SIRT1 expression is significantly associated with CIMP-high MSI-high status, particularly in the presence of *BRAF* mutation. Our data also indicate that SIRT1 is related with DNA methylation in gene-specific CpG islands, rather than global DNA methylation level. Considering that SIRT1 is a promising target of chemotherapy and chemoprevention, our findings may have considerable clinical implications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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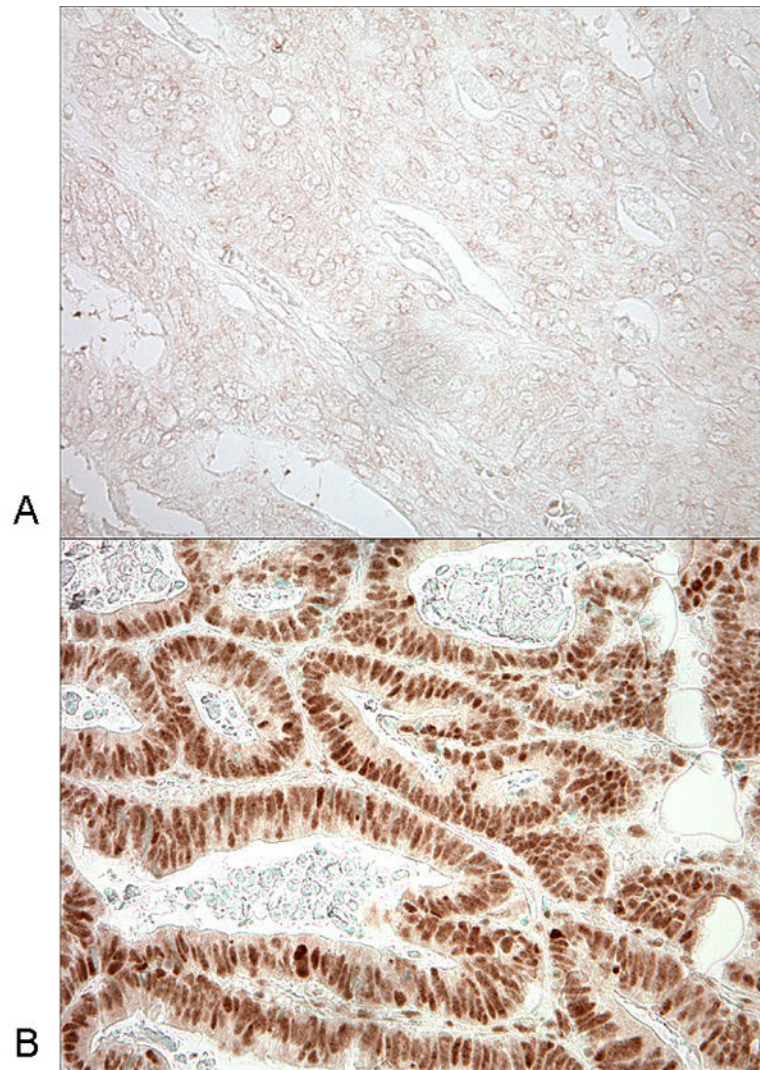
Figure 1.
SIRT1 expression in colorectal cancer.

A. No overexpression of SIRT1 in colon cancer cells. B. Overexpression of SIRT1 in nuclei of colorectal cancer cells.
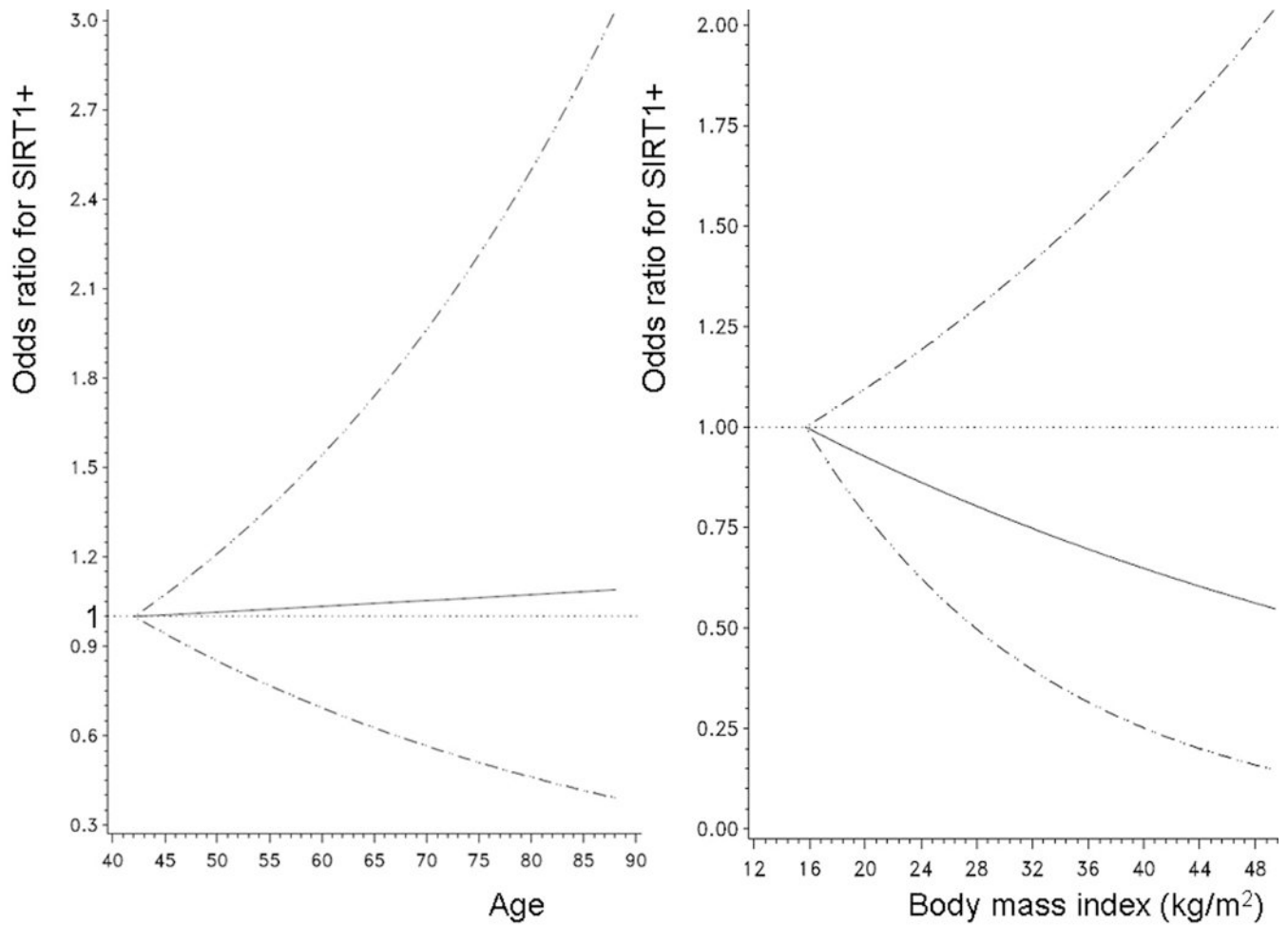
Figure 2.
Smoothing spline plots for the relations between age and SIRT1 (left panel) and between body mass index and SIRT1 (right panel).

Unadjusted odds ratio for the association with SIRT1+ is shown as young age (left panel) or low body mass index (right panel) as a referent. 95% confidence interval is indicated by hatched lines.
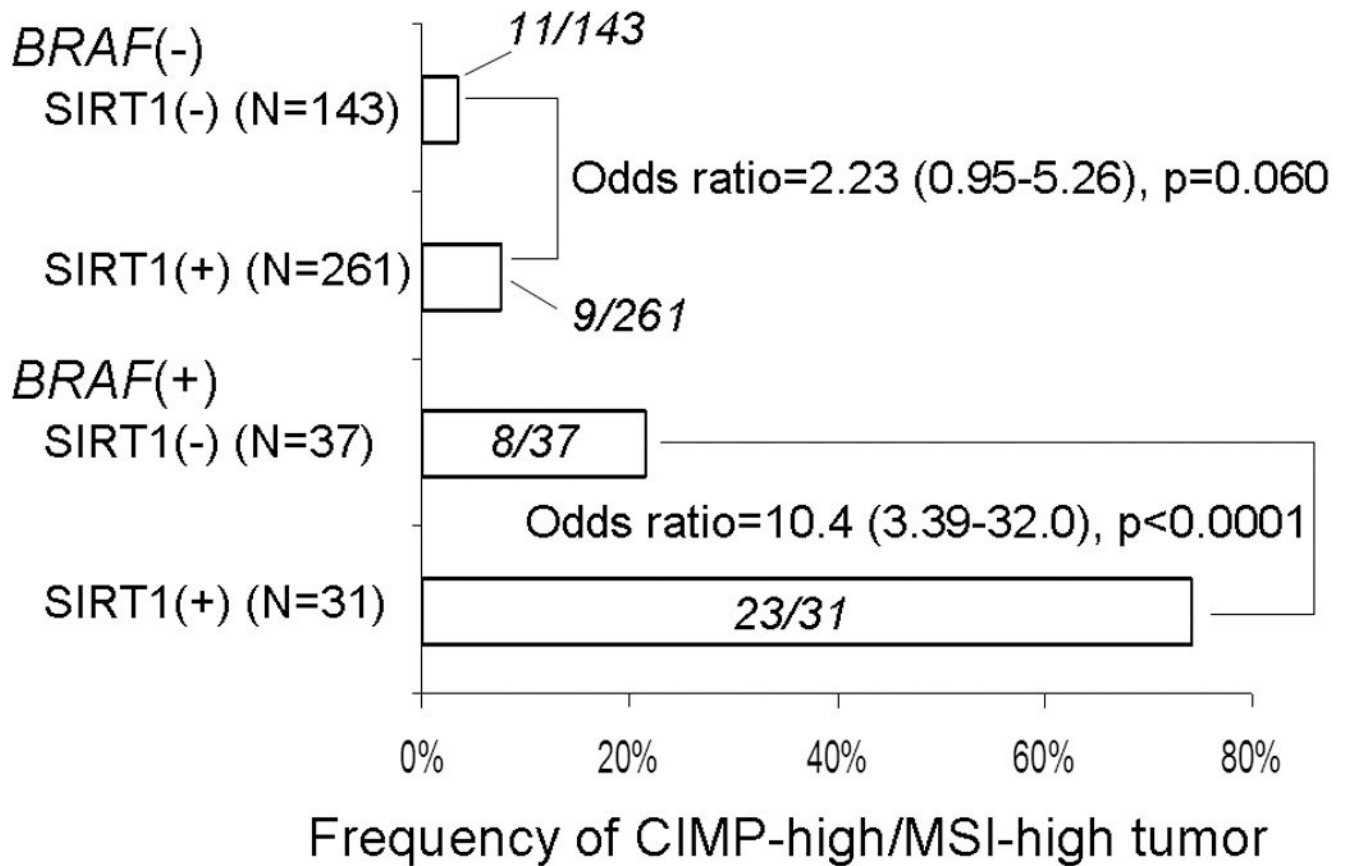
Figure 3.
Frequency of the CIMP-high/MSI-high phenotype colorectal cancers stratified by SIRT1
and *BRAF* status.

Table 1

Frequency of SIRT1 overexpression in colorectal cancer

Clinical or pathologic feature	Total N	SIRT1+	Odds ratio (95% confidence interval)	P value
All cases	485	180 (37%)		
Gender				
Men	194	64 (33%)	1	
Women	291	116 (40%)	1.35 (0.92–1.97)	
Age				
59	125	45 (36%)	1	
60–69	207	78 (38%)	1.07 (0.68–1.70)	
70	153	57 (37%)	1.06 (0.65–1.72)	
Body mass index (kg/m^2)				
<25	199	74 (37%)	1	
25–30	172	66 (38%)	1.05 (0.69–1.60)	
30	87	30 (34%)	0.89 (0.52–1.51)	
Tumor location				
Distal (splenic flexure to rectum)	233	79 (34%)	1	
Proximal (cecum to transverse colon)	234	92 (39%)	1.26 (0.87–1.84)	
Stage				
I	97	35 (36%)	1	
II	147	61 (42%)	1.26 (0.74–2.13)	
III	132	44 (33%)	0.89 (0.51–1.54)	
IV	69	25 (36%)	1.01 (0.53–1.91)	
Tumor grade				
Low	422	149 (35%)	1	Referent
High	47	27 (57%)	2.47 (1.34–4.56)	0.003
Mucinous component				
0%	261	88 (34%)	1	Referent
1–49%	106	46 (43%)	1.51 (0.95–2.39)	0.08
50%	65	31 (48%)	1.79 (1.03–3.11)	0.04
Signet ring cell component				

Clinical or pathologic feature	Total N	SIRT1+	Odds ratio (95% confidence interval)	P value
0%	453	168 (37%)	1	
1%	32	12 (38%)	1.02 (0.49–2.13)	

Only significant p values are described.

Table 2

Frequency SIRT1 overexpression in colorectal cancer according to various molecular features

Molecular feature	Total N	SIRT1+	Odds ratio (95% confidence interval)	P value
CIMP status (No. of methylated CIMP markers)				
CIMP-0 (0)	209	76 (36%)	1	Referent
CIMP-low (1–5)	187	58 (31%)	1.27 (0.84–1.93)	
CIMP-high (6–8)	74	42 (57%)	2.30 (1.34–3.94)	0.002
MSI status				
MSS	345	117 (34%)	1	Referent
MSI-low	55	14 (25%)	1.50 (0.79–2.87)	
MSI-high	83	49 (59%)	2.81 (1.72–4.59)	<0.0001
CIMP and MSI status				
CIMP-low/0 MSI-low/MSS	367	121 (33%)	1	Referent
CIMP-high MSI-low/MSS	22	7 (32%)	0.95 (0.38–2.39)	
CIMP-low/0 MSI-high	28	13 (46%)	1.76 (0.81–3.82)	
CIMP-high MSI-high	52	35 (67%)	4.19 (2.25–7.77)	<0.0001
BRAF mutation				
(−)	404	143 (35%)	1	
(+)	68	31 (46%)	1.53 (0.91–2.57)	
KRAS mutation				
(−)	310	120 (39%)	1	
(+)	173	59 (34%)	0.82 (0.56–1.21)	
PIK3CA mutation				
(−)	374	137 (37%)	1	
(+)	57	23 (40%)	1.17 (0.66–2.07)	
LINE-1 methylation				
70%	58	22 (38%)	1	
60–70%	155	61 (39%)	1.06 (0.57–1.98)	
50–60%	184	72 (39%)	1.05 (0.57–1.93)	
<50%	66	20 (30%)	0.71 (0.34–1.50)	

Molecular feature	Total N	SIRT1+	Odds ratio (95% confidence interval)	P value
p53 *				
(−)	285	110 (39%)	1	
(+)	197	69 (35%)	0.86 (0.59–1.25)	
Nuclear β-catenin *				
(−)	269	104 (39%)	1	
(+)	166	68 (41%)	1.10 (0.74–1.63)	
FASN (fatty acid synthase) *				
(−)	427	149 (35%)	1	Referent
(+)	52	28 (54%)	2.18 (1.22–3.89)	0.008
COX-2 (cyclooxygenase-2) *				
(−)	92	32 (35%)	1	
(+)	391	147 (38%)	1.13 (0.70–1.82)	

Only significant p values are described.

*
p53, β-catenin, COX-2 and FASN were assessed by immunohistochemistry.

Table 3

Multivariate analysis of the relations with SIRT1 in colorectal cancer

Variable independently associated with SIRT1	Multivariate odds ratio (95% confidence interval)	P value
CIMP-high MSI-high (vs. all other MSI CIMP subtypes)	3.20 (1.35–7.59)	0.008
Any mucinous component (vs. 0% mucin)	1.86 (1.15–3.01)	0.01
High tumor grade (vs. low grade)	2.71 (1.19–6.15)	0.02
FASN (fatty acid synthase)	1.95 (1.03–3.69)	0.04

Multivariate logistic regression analysis assessing the relations with SIRT1 included age, sex, body mass index, tumor location, stage, grade, mucin, signet ring cells, MSI/CIMP subtype, p53, FASN, COX-2, β-catenin, LINE-1, *KRAS, PIK3CA* and *BRAF*. Only significant variables are listed.

Table 4

SIRT1 expression and patient mortality in colorectal cancer

	Total N	Cancer-specific mortality				Overall mortality			
		Deaths / person-years	Univariate hazard ratio (95% confidence interval)	Stage-matched hazard ratio (95% confidence interval)	Multivariate hazard ratio (95% confidence interval)	Deaths / person-years	Univariate hazard ratio (95% confidence interval)	Stage-matched hazard ratio (95% confidence interval)	Multivariate hazard ratio (95% confidence interval)
Colon and rectum									
SIRT1 (−)	286 (63%)	76/2341	1 (referent)	1 (referent)	1 (referent)	130/2341	1 (referent)	1 (referent)	1 (referent)
SIRT1 (+)	170 (37%)	40/1211	0.91 (0.62–1.34)	0.94 (0.63–1.39)	0.98 (0.61–1.59)	70/1211	1.03 (0.77–1.38)	1.02 (0.76–1.37)	1.07 (0.76–1.52)
Colon									
SIRT1 (−)	229 (61%)	57/1924	1 (referent)	1 (referent)	1 (referent)	103/1924	1 (referent)	1 (referent)	1 (referent)
SIRT1 (+)	147 (39%)	36/1043	1.02 (0.67–1.54)	0.99 (0.64–1.53)	1.18 (0.69–2.00)	62/1043	1.10 (0.80–1.52)	1.06 (0.76–1.47)	1.20 (0.82–1.75)

The multivariate, stage-matched conditional Cox model included age, year of diagnosis, sex, body mass index, tumor location, stage, grade, *KRAS*, *BRAF*, *PIK3CA*, p53, COX2, FASN, β-catenin, LINE-1 methylation, microsatellite instability, and the CpG island methylator phenotype.