Significant Association of Oncogene YAP1 with Poor Prognosis and Cetuximab Resistance in Colorectal Cancer Patients

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Abstract

Purpose: Activation of YAP1, a novel oncogene in the Hippo pathway, has been observed in many cancers, including colorectal cancer. We investigated whether activation of YAP1 is significantly associated with prognosis or treatment outcomes in colorectal cancer.

Experimental Design: A gene expression signature reflecting YAP1 activation was identified in colorectal cancer cells, and patients with colorectal cancer were stratified into two groups according to this signature: activated YAP1 colorectal cancer (AYCC) or inactivated YAP1 colorectal cancer (IYCC). Stratified patients in five test cohorts were evaluated to determine the effect of the signature on colorectal cancer prognosis and response to cetuximab treatment.

Results: The activated YAP1 signature was associated with poor prognosis for colorectal cancer in four independent patient cohorts with stage I–III disease (total $n = 1,028$). In

a multivariate analysis, the impact of the YAP1 signature on disease-free survival was independent of other clinical variables [hazard ratio (HR), 1.63; 95% confidence interval (CI), 1.25–2.13; $P < 0.001$]. In patients with stage IV colorectal cancer and wild-type KRAS, IYCC patients had a better disease control rate and progression-free survival (PFS) after cetuximab monotherapy than did AYCC patients; however, in patients with KRAS mutations, PFS duration after cetuximab monotherapy was not different between IYCC and AYCC patients. In multivariate analysis, the effect of YAP1 activation on PFS was independent of KRAS mutation status and other clinical variables (HR, 1.82; 95% CI, 1.05–3.16; $P = 0.03$).

Conclusions: Activation of YAP1 is highly associated with poor prognosis for colorectal cancer and may be useful in identifying patients with metastatic colorectal cancer resistant to cetuximab. *Clin Cancer Res; 21(2); 357–64. ©2014 AACR.*

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Introduction

Colorectal cancer is a major contributor to cancer mortality and morbidity in developed countries and is the second leading cause of cancer deaths in the United States (1). Current prognostic models use histoclinical parameters for prognostication of individual patients but have limitation in capturing molecular heterogeneity of this disease. Recent studies identified several molecular subtypes of colorectal cancer reflecting molecular heterogeneity of colorectal cancer by using various methods of screening cancer genome (2–6). However, the biologic characteristics of these subtypes are poorly understood, and the responses of these subtypes to specific treatments are unknown.

The Hippo pathway is a novel tumor suppressor pathway that is well conserved in different species (7, 8). When Hippo signaling is active, its downstream oncogene YAP1 and the related TAZ are phosphorylated and inactivated by the Hippo core complex. When Hippo signaling is absent or suppressed, however, unphosphorylated YAP1 and TAZ enter the nucleus and induce transcription of genes involved in cell proliferation and survival. Deregulation of YAP1 and TAZ has been discovered in various human cancers, including colorectal cancer (9–16).

YAP1 and TAZ play important roles in the development of colorectal cancer as evidenced by their overexpression in colorectal cancer (7, 8, 10, 11, 16) which promotes proliferation and survival of colorectal cancer cells (7, 17). However, despite

Translational Relevance

Colorectal cancer is a clinically heterogeneous disease. Previous studies suggested potential molecularly distinct subtypes of colorectal cancer. However, the biologic characteristics of these subtypes are poorly understood, and the responses of these subtypes to specific treatments are unknown. In this study, we were able to subdivide patients with colorectal cancer into two major subgroups that are characterized by activation of oncogene YAP1 and showed significant differences in disease-free survival. Furthermore, we also demonstrated that the YAP1 activation is significantly associated with lack of response to cetuximab monotherapy. Most interestingly, among patients with wild-type KRAS, only patients without YAP1 activation benefited from cetuximab treatment. This study provides a strong rationale for evaluating the status of YAP1 activation as potential prognostic and predictive markers in future studies. This result might improve patient care by providing more practical guidance for different treatments.

increasing evidence supporting the involvement of YAP1 and TAZ in colorectal cancer progression, the clinical relevance of YAP1 activation has yet to be properly examined in colorectal cancer. In the present study, we systematically characterized genomic data from multiple cohorts of patients with colorectal cancer to determine the clinical significance of YAP1 activation in colorectal cancer cells. This approach led to the development of molecular signatures by which patients can be stratified according to activation of YAP1. Further analysis of the data revealed that YAP1 activation is closely associated with resistance of colorectal cancer to treatment with cetuximab.

Materials and Methods

Cell culture and generation of YAP1 signatures in colorectal cancer cells

The colorectal cancer cell line NCI-H716 was purchased from the ATCC and cultured as suggested by the supplier. A constitutively active mutant of human YAP1 (YAP1-S127A) that was described previously (18) was obtained from Addgene, a non-profit organization for sharing plasmids (www.addgene.org). YAP1-S127A was expressed in NCI-H716 cells by using lentiviral vector containing YAP1-S127A coding sequence; an empty lentivirus was used as a control (mock). Overexpression of YAP1-S127A in transfected cells was confirmed via Western blotting with a mouse polyclonal antibody against human YAP1 (1:500 dilution; Santa Cruz Biotechnology; Supplementary Fig. S1). Total RNA was extracted from NCI-H716 cells expressing exogenous YAP1-S127A and used for labeling and hybridization to human expression BeadChips (HumanHT-12 v4 Expression BeadChip Kit; Illumina) according to the manufacturer's protocols. Untransfected and empty vector-transfected NCI-H716 cells were used as controls. All experiments were performed in triplicate. For validation of YAP1-specific signature from NCI-H716 cells, we generated additional gene expression data from MKN45 cells overexpressing same exogenous YAP1-S127A via lentiviral vector. MKN45 cells were selected because it has the lowest basal level expression of YAP1 due to deletion of both alleles of YAP1 gene (14). Primary microarray data from both cell lines are

available in the National Cancer for Biotechnology Information Gene Expression Omnibus (GEO) database (GSE41387, GSE50490). For further independent validation of YAP1 signature from NCI-H716 cells, gene expression data from MCF10A cells were downloaded and processed from GEO (GSE13861 and GSE26942).

Patient and genomic data

We assembled a multistudy microarray database of colorectal cancer expression profiles (total $n = 1,108$) based on the Affymetrix U133 GeneChip microarray platform. The database encompasses five different colorectal cancer cohorts for which corresponding microarray data and clinical annotations were extracted from the GEO public data repository. Cohort 1 consisted of patients with colorectal cancer with stage I–III disease ($n = 229$) whose fresh-frozen tumor specimens had been retrieved from the tissue banks of the Royal Melbourne Hospital (Parkville, Victoria, Australia) and the H. Lee Moffitt Cancer Center and Research institute (Tampa, FL; GEO accession number GSE14333; ref. 2). Cohort 2 was composed of 168 patients with colorectal cancer with stage I–III disease whose data had been generated from fresh-frozen tumor specimens at the Institut National de la Santé et de la Recherche Médicale (Paris, France; GEO accession number GSE37892) and the Vanderbilt University Medical Center (Nashville, TN; GEO accession number GSE17538; ref. 19). Cohort 3 was made up of 506 patients with colorectal cancer with stage I–III disease from a French multicenter study (GEO accession number GSE39582; ref. 3). The disease-free survival (DFS) duration was defined in the previous studies as the time from surgery to the first documented recurrence or death of colorectal cancer (2, 3, 19). Cohort 4 consisted of 125 patients with colorectal cancer patients with stage I–III disease whose microarray data were generated from analysis of fresh-frozen tumor tissue at Memorial Sloan-Kettering Cancer Center (New York, NY; GEO accession number GSE41258; ref. 20). In that study, the cancer-specific survival (CSS) duration was defined as the time from surgery to a documented colorectal cancer–related death. The data for cohort 5 were composed of patients with refractory metastatic colorectal cancer who received cetuximab monotherapy in a clinical trial (21). Of 110 patients who participated in the trial, 80 patients with tumor mRNA expression data (GEO accession number GSE5851; ref. 21) were included in this cohort. In the study of this cohort, patient characteristics were presented, and the progression-free survival (PFS) duration was defined as the time from study enrollment to disease progression or death (21). KRAS mutation status in cohort 5 was determined by direct sequencing of PCR-amplified exon 2 genomic region of KRAS in previous study (21).

Gene expression data for a sixth cohort were downloaded from The Cancer Genome Atlas (TCGA; http://cancergenome.nih.gov). The full data for this cohort were described in detail previously (6). Comprehensive genetic information on 195 patients with colorectal cancer in this cohort was available.

Statistical analyses of microarray data

The BRB-ArrayTools software program (http://linus.nci.nih.gov/BRB-ArrayTools.html) was used for analysis of gene expression data (22). Other statistical analyses were performed in the R language (http://www.r-project.org) or using the SPSS statistical software program (version 21; IBM Corporation). Raw data on the patient cohorts were downloaded from the GEO database and

normalized using a robust multiarray averaging method (23, 24). Genes that were differentially expressed in three groups of NCI-H716 cell lines and related to YAP1 activation were identified using a t test. Differences in gene expression among the three sample groups were considered statistically significant if the P value was less than 0.005. A heatmap was generated using the Cluster and TreeView software programs (25).

To predict a class of individual patients in the six cohorts, a previously developed approach was used (4, 26–28). Matched probes to 199 genes were selected from each dataset from patients: 174 probes for cohort 1, 2, and 3 datasets (Affymetrix U133 version 2) and 142 probes for cohort 4 and 5 datasets (Affymetrix U133). Briefly, gene expression data in the training set (the YAP1 signature in NCI-H716 cells) were combined to form a classifier according to a Bayesian compound covariate predictor (BCCP; 29). The robustness of the classifier was estimated using a misclassification rate determined during leave-one-out cross-validation in the training set. The BCCP classifier estimated the likelihood that an individual patient had either an activated YAP1 signature—activated YAP1 colorectal cancer (AYCC)—or an inactivated YAP1 signature—inactivated YAP1 colorectal cancer (IYCC). After the BCCP classifier was used to dichotomize the patients according to the YAP1 signature, the prognostic significance was estimated using Kaplan–Meier plots (log-rank tests). Multivariate Cox proportional hazards regression analysis was used to evaluate the effect of YAP1 signature on survival independently of other clinical parameters; the parameters included in the multivariate analyses are presented in individual tables or Supplementary Tables.

In cohort 5, differences in response of colorectal cancer to treatment of cetuximab were verified using χ^2 tests. P values less than 0.05 were considered statistically significant.

Results

Correlation of YAP1 signature with clinical characteristics

Systematic comparison of the gene expression data for the three groups of NCI-H716 colorectal cancer cells identified a YAP1-specific gene expression signature comprising 199 unique genes (Supplementary Figs. S1 and S2 and Supplementary Table S1). Expression of *CTGF*, one of the best known direct downstream targets of *YAP1*, was highly upregulated in *YAP1*-overexpressing NCI-H716 cells, providing additional confirmation that modulation of gene expression in the cells is due to activation of *YAP1*. To ensure authenticity of YAP1 signature, we compared 199-gene signature to gene expression data from two independent cell lines overexpressing YAP1 by using gene set enrichment approach. Vast majority of 199 genes were significantly enriched in YAP1-over-expressing MKN45 and MCF10A cells (Supplementary Fig. S3), suggesting that majority of 199 genes are direct or indirect targets of YAP1.

To examine the correlation of YAP1 activation with clinical characteristics of colorectal cancer, we compared the 199-gene YAP1 signature with gene expression data for patients with colorectal cancer. Specifically, we used the BCCP algorithm to calculate the probability of YAP1 activation in colorectal cancer tissue specimens (Fig. 1A). We found that 14.9% to 39.3% of the patients in the six cohorts had the activated YAP1 signature (AYCC group; Table 1). Also, we analyzed the correlation of clinical characteristics with the YAP1 signature in five of the

cohorts (we excluded cohort 5, which consisted of patients with stage IV colorectal cancer). Although AYCC patients had slightly more advanced disease than did IYCC patients in cohort 2 ($P = 0.023$), we did not see a clear difference in stage distribution between the AYCC and IYCC groups in the other four cohorts. In addition, we found no differences in other clinical variables between the IYCC and AYCC groups (Supplementary Tables S2–S6).

### Prognostic impact of YAP1 activation

We next investigated the prognostic impact of YAP1 activation using data on patients with stage I–III colorectal cancer (cohorts 1–4). Tumor recurrence and DFS data were available for cohorts 1 to 3, but we had to analyze CSS data for cohort 4 as DFS data for that group were not available. Kaplan–Meier curves for these patients consistently demonstrated much worse survival durations in AYCC patients than in IYCC patients (Fig. 1B–E), indicating that the activated YAP1 signature is clearly related to poor prognosis for colorectal cancer.

We conducted further analyses to determine whether the prognostic impact of the YAP1 signature is independent of other clinical variables. We pooled the patients in cohorts 1 to 3 with available DFS data ($n = 903$) for univariate and multivariate analyses of factors affecting DFS (Table 2). In the multivariate analysis, AYCC was related to worse DFS rates than was IYCC [HR, 1.63; 95% confidence intervals (CI), 1.25–2.13; $P < 0.001$] independent of other clinical variables. When we conducted the same analyses independently after splitting the group into two (cohorts 1 and 2 vs. cohort 3, as cohort 3 had more detailed clinical variables than did the other two cohorts), the independent impact of the YAP1 signature on DFS remained unchanged (Supplementary Tables S7 and S8). Furthermore, the activated YAP1 signature successfully identified patients with poor prognosis regardless of their disease stage ($P < 0.05$; Supplementary Fig. S4). Taken together, these findings suggested that the prognostic relevance of the YAP1 signature to patients with colorectal cancer is maintained even when taking into account the classic clinicopathological prognostic features.

YAP1 activation is associated with poor response to cetuximab treatment

All of the patients in cohort 5 ($n = 80$) received cetuximab monotherapy. In the 70 patients in this cohort who had KRAS mutation status data available, we observed no difference in the KRAS mutation rates between the AYCC and IYCC groups (Supplementary Table S9). However, we did see differences in response to cetuximab between the two groups. Specifically, tumor shrinkage (complete remission or partial remission) occurred only in the IYCC group [response rate: 10.5% (IYCC) vs. 0.0% (AYCC); $P = 0.175$], and the disease control rate was significantly higher in the IYCC group than in the AYCC group (38.6% vs. 13.0%; $P = 0.026$). As expected, patients with wild-type (WT) KRAS had a longer PFS duration than did patients with KRAS mutations in this cohort (21), although it did not reach statistical significance ($P = 0.142$, Supplementary Fig. S5). However, IYCC patients had a significantly longer PFS duration than did AYCC patients ($P = 0.005$; Fig. 2A), more so in WT KRAS patients ($P = 0.015$; Fig. 2B) than in KRAS-mutant patients ($P = 0.396$; Fig. 2C). In multivariate analysis, the effect of the YAP1 signature on PFS according to other clinical variables was unchanged (Table 3).
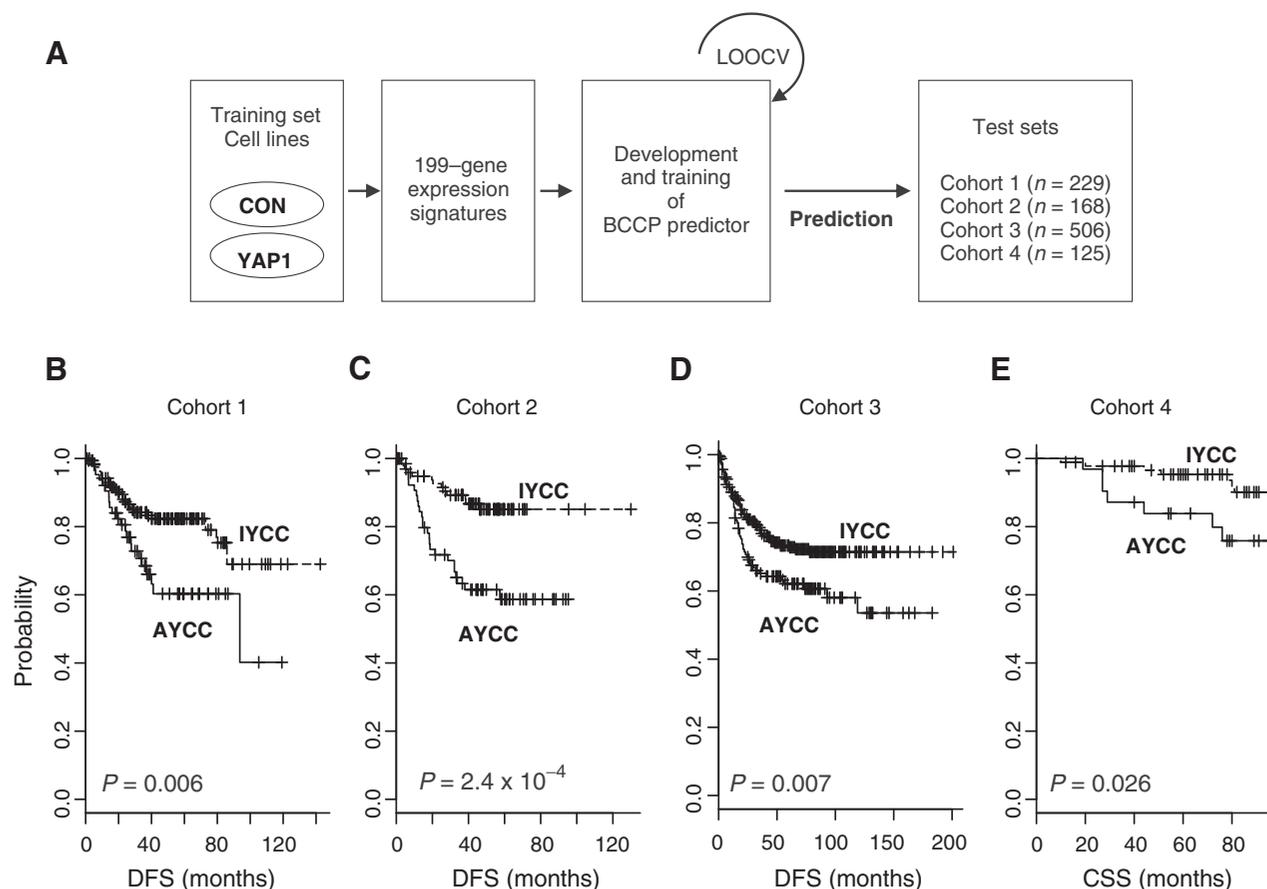
Figure 1.
Construction of a prediction model using gene expression profiles for NCI-H716 cells and analyses of survival in patient cohorts 1 to 4. A, schematic of the strategy used to construct the prediction model and evaluate predicted colorectal cancer outcomes according to gene expression signature. CON, control. B–E, Kaplan–Meier plots of the AYCC and IYCC patients in cohorts 1 to 4. *P* values were calculated using log-rank tests. +, censored data.

Relationship between the YAP1 signature and other genetic events

We further investigated the correlation between the YAP1 signature and somatic mutations in patients in cohort 6, as they had the most comprehensive genetic information, which was available from the TCGA database (Fig. 3 and Supplementary Table S6). When the same genetic data are available from other cohorts, analyzed results were compared among the cohorts. For cohort 6, we compared the mutational statuses of genes involved in five pathways (WNT, MAPK, PI3K, TGFβ, and p53), which are reported to be deregulated in colorectal cancer cells (6). Of the 30 compared genes, only ACVR2A exhibited a significantly different mutation rate between the IYCC and AYCC groups (12% vs. 0%; $P = 0.048$; Fig. 3 and Supplementary Table S6). ACVR2A is activin receptor type-2A, a member of TGFβ superfamily (30). It is interesting to point out that ACVR2A is also frequently mutated gene in stomach cancer (31), suggesting that it may play critical roles in development of gastrointestinal cancer. We observed no differences in mutation rate between the AYCC and IYCC groups for other genes, including KRAS (cohorts 3, 5, and 6), BRAF (cohorts 3 and 6), and TP53 (cohorts 3 and 6; Supplementary Tables S4, S6, and S9). Most interestingly, hypermutated tumors, which were defined by the TCGA project (6), developed more frequently in the IYCC group than in the AYCC group ($P = 0.05$).

The higher mutation rate in the IYCC group was supported by a higher frequency of nonsilent gene mutations (per 10^6 bases) in the IYCC group than in the AYCC group ($P = 0.002$, Mann–Whitney U test).

Discussion

In this study, we extracted the YAP1 gene activation signature from colorectal cancer cell microarray data and classified patients with colorectal cancer into two groups: AYCC and IYCC. Among four independent cohorts of patients with locoregional colorectal cancer, we found that AYCC patients had worse survival rates than did IYCC patients. Also, among those with stage IV colorectal cancer, we showed that the YAP1 signature is associated with response to cetuximab monotherapy, generating interesting hypothesis connecting YAP1 activation to potential benefit of cetuximab treatment. To our knowledge, this is the first study to suggest that the YAP1 gene signature can be used as prognostic biomarker in locoregional colorectal cancer and as potential predictive biomarker in patients with metastatic colorectal cancer receiving cetuximab treatment.

Previous studies examined the prognostic relevance of YAP1 or TAZ activation in patients with colorectal cancer, but their results were inconsistent because the investigators used different

Table 1. Patient characteristics in 6 patient cohorts

	Cohort 1 (*N* = 229)	Cohort 2 (*N* = 168)	Cohort 3 (*N* = 506)	Cohort 4 (*N* = 125)	Cohort 5 (*N* = 80)	Cohort 6 (*N* = 195)
Gender						
Male	123 (53.7%)	90 (53.6%)	279 (55.1%)	63 (50.4%)	44 (55.0%)	103 (52.8%)
Female	106 (46.3%)	78 (46.4%)	227 (44.9%)	62 (49.6%)	36 (45.0%)	92 (47.2%)
Age, years						
Median (range)	67 (26–92)	67 (22–97)	68 (22–97)	68 (23–87)	60.5 (25–89)	69 (35–90)
Location						
Right colon	102 (44.5%)	57 (33.9%)	206 (40.7%)	50 (40.0%)	NA	67 (34.4%)
Left colon	95 (41.5%)	72 (42.9%)	300 (59.3%)	65 (52.0%)	NA	65 (33.3%)
Rectum	30 (13.1%)	0 (0.0%)	0 (0.0%)	10 (8.0%)	NA	62 (31.8%)
Unknown	2 (0.9%)	39 (23.2%)	0 (0.0%)	0 (0.0%)	NA	1 (0.5%)
Stage						
I	44 (19.2%)	4 (2.4%)	37 (7.3%)	28 (22.4%)	0 (0.0%)	43 (22.1%)
II	94 (41.0%)	88 (52.4%)	264 (52.2%)	48 (38.4%)	0 (0.0%)	72 (36.9%)
III	91 (39.7%)	76 (45.2%)	205 (40.5%)	49 (39.2%)	0 (0.0%)	49 (25.1%)
IV	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	80 (100.0%)	29 (14.9%)
Unknown	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.0%)
Adjuvant chemotherapy						
Yes	87 (38.0%)	23 (13.7%)	203 (40.1%)	NA	NA	NA
No	142 (62.0%)	15 (8.9%)	301 (59.5%)	NA	NA	NA
Unknown	0 (0.0%)	130 (77.4%)	2 (0.4%)	NA	NA	NA
KRAS status						
WT	NA	NA	297 (58.7%)	NA	43 (53.8%)	115 (59.0%)
Mutant	NA	NA	188 (37.2%)	NA	27 (33.8%)	80 (41.0%)
Unknown	NA	NA	21 (4.2%)	NA	10 (12.5%)	0 (0.0%)
MSI status						
pMMR	NA	NA	388 (76.7%)	87 (69.6%)	NA	172 (88.2%)
dMMR	NA	NA	73 (14.4%)	28 (22.4%)	NA	23 (11.8%)
Unknown	NA	NA	45 (8.9%)	10 (8.0%)	NA	0 (0.0%)
YAP1 signature						
IYCC	163 (71.2%)	102 (60.7%)	366 (72.3%)	92 (73.6%)	57 (71.3%)	166 (85.1%)
AYCC	66 (28.8%)	66 (39.3%)	140 (27.7%)	33 (26.4%)	23 (28.8%)	29 (14.9%)

Abbreviations: MSI, microsatellite instability; pMMR, proficient mismatch repair; dMMR, deficient mismatch repair; NA, not available.

methods of measuring YAP1 signal activation (11, 16, 32, 33). Wang and colleagues (16) measured protein expression of YAP1 in colorectal cancer to estimate YAP1 activity. However, because YAP1 is largely regulated by phosphorylation (8), this approach may not be able to fully measure YAP1 activity in colorectal cancer. In addition, Yuen and colleagues (33) reported that the TAZ mRNA expression level was a prognostic indicator but that YAP1 mRNA expression was not related to prognosis. However, because both YAP and TAZ exist as nuclear or cytoplasmic form and activated YAP1 and TAZ function mainly in the nucleus, the

Table 2. Univariate and multivariate analyses of factors affecting DFS in stage I–III patients (patient data from cohorts 1, 2, and 3 were pooled together; *N* = 903)

	Univariate analysis[a]			Multivariate analysis[b]	
Variables	Patients (*n*)	5-Year DFS	*P*	HR (95% CI)	*P*
Age[c]					
<70 y	475	70.1%	—	—	—
≥70 y	427	75.3%	0.221	—	—
Gender					
Male	492	69.3%	—	1.00 (—)	—
Female	411	76.0%	0.086	0.80 (0.61–1.05)	0.105
Location			0.014		0.070
Right colon	365	73.3%	—	1.00 (—)	—
Left colon	467	69.4%	—	1.18 (0.90–1.56)	0.228
Rectum	30	77.5%	—	1.23 (0.56–2.67)	0.606
Unknown	41	97.3%	—	0.11 (0.02–0.76)	0.026
Adjuvant chemotherapy			<0.001		0.859
Undone	458	78.0%	—	1.00 (—)	—
Done	313	66.6%	—	0.94 (0.68–1.30)	0.716
Unknown	132	67.9%	—	0.90 (0.60–1.34)	0.593
Stage			<0.001		<0.001
I	85	95.7%	—	1.00 (—)	—
II	446	79.7%	—	4.80 (1.50–15.29)	0.008
III	372	59.0%	—	11.88 (3.69–38.26)	<0.001
YAP1 status					
IYCC	631	77.5%	—	1.00 (—)	—
AYCC	272	61.2%	<0.001	1.63 (1.25–2.13)	<0.001

[a]In univariate analyses, log-rank tests were conducted.
[b]In the multivariate Cox proportional hazard model, only variables with *P* < 0.15 in univariate analysis were included and the "enter method" was applied.
[c]Data on age of one patient were missing.
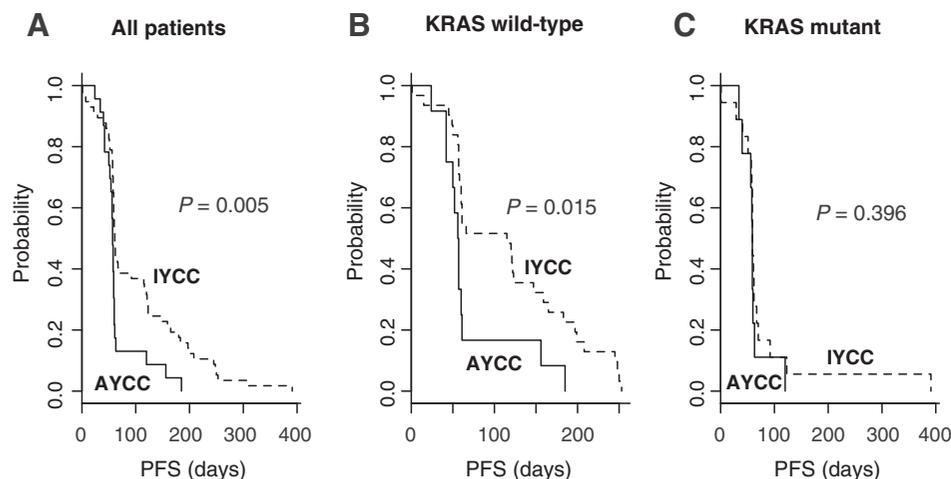
Figure 2.
PFS according to YAP1 signature in (A) all patients ($n = 80$), (B) patients with WT KRAS ($n = 43$), and (C) patients with mutant KRAS ($n = 27$) in cohort 5. KRAS mutation data were not available for 10 patients.

total expression level cannot properly reflect the true biologic activity of these proteins. Moreover, Barry and colleagues (32) recently suggested that cytoplasmic YAP1 may have a growth-inhibitory function in colorectal cancer cells. Therefore, if YAP1 (or TAZ) is to be used as a biomarker, both the level of expression and intracellular location of this protein must be considered. Alternatively, expression patterns for downstream genes targeted by YAP1 or TAZ in colorectal cancer cells may be used as indicators of their activation because their best known molecular activity is transcription activation (7, 8). Because the cutoff point or criterion for YAP1 and TAZ activation, which is measured using mRNA or IHC, has gone unverified until now, we developed the YAP1 signature, which reflects the transcriptional activity of YAP1 in colorectal cancer cells. In addition, unlike previous studies that did not examine patients with colorectal cancer according to disease stage (11, 16, 32, 33), we categorized patients into two classes—locoregional (stage I–III) and remote (stage IV) disease—because the therapeutic approaches for these two classes are clearly different. Using the YAP1 signature and data from four large cohorts of patients with colorectal cancer ($n = 1,028$), we found that the activated YAP1 signature is independently

predictive of poor survival in patients with stage I–III disease (Fig. 1).

Treatment with cetuximab, a monoclonal antibody against EGFR, is effective against metastatic colorectal cancer, but its beneficial effect is limited to patients with WT KRAS (34, 35). However, even in patients with WT KRAS, the benefit of treatment with cetuximab is restricted to a small proportion of patients and is not sustainable (35). Therefore, selection of patients with metastatic colorectal cancer who would have the maximum benefit of this treatment is important (21, 36, 37). In the present study, using genomic data for patients with colorectal cancer enrolled in a clinical trial (21), we showed that YAP1 activation is significantly associated with poor response to cetuximab therapy in colorectal cancer (Fig. 2). This observation is in good agreement with previous report showing that silencing of YAP1 sensitized ovarian cancer cells to EGFR inhibitor erlotinib (38). Furthermore, recent study also identified YAP1 as a potential biomarker for cetuximab resistance in head and neck cancer (39).

This study had a few limitations. First, our observations must be validated in prospective studies. The fact that we obtained the same results for four independent patient cohorts strongly

Table 3. Univariate and multivariate analyses of factors affecting PFS in patients who received cetuximab monotherapy (cohort 5)

Variables	Patients (*n*)	PFS (median, d)	6-Month progression-free rate (%)	*P*	HR (95% CI)	*P*
		Univariate analysis[a]			**Multivariate analysis[b]**	
Age[c]						
<70 y	54	59	18.5%	—	—	—
≥70 y	24	60	8.3%	0.227	—	—
Gender						
Male	44	61	22.7%	—	1.00 (—)	—
Female	36	58	5.6%	0.009	1.65 (0.98–2.78)	0.062
KRAS status[d]						
WT	43	61	20.9%	—	1.00 (—)	—
Mutant	27	59	3.7%	0.142	1.27 (0.75–2.14)	0.375
YAP1 status						
IYCC	57	61	19.3%	—	1.00 (—)	—
AYCC	23	57	4.3%	0.005	1.82 (1.05–3.16)	0.034

[a]In univariate analyses, log-rank tests were conducted.
[b]In the multivariate Cox proportional hazard model, only variables with $P < 0.15$ in univariate analysis were included and the "enter method" was applied. In this multivariate analysis, 70 patients from cohort 6 without missing data on KRAS status were included.
[c]Data on age of 2 patients were missing.
[d]Data on KRAS mutational status of 10 patients were missing.
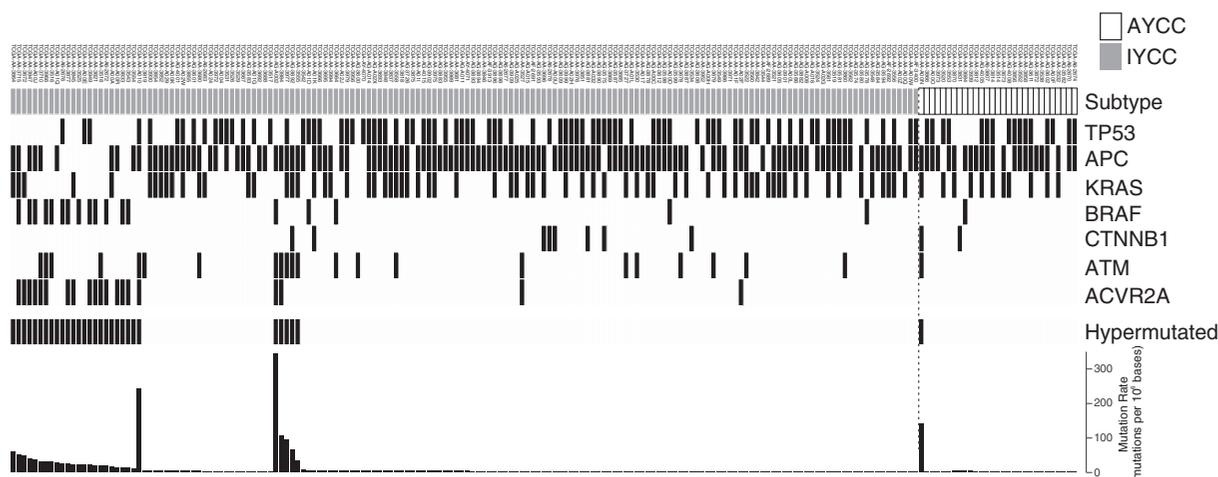
Figure 3.
Genetic changes in colorectal cancer cells according to YAP1 signature in cohort 6. Genetic data were retrieved from the TCGA database and analyzed.

suggests that the YAP1 signature is a reliable tool for assessing prognosis for locoregional colorectal cancer. We also demonstrated the possibility of using the YAP1 signature as a predictive marker for response of colorectal cancer to treatment with cetuximab. However, because we applied the developed YAP1 signature to retrospective patient cohorts, our observations require validation. Also necessary is investigating whether the efficacy of other chemotherapeutic agents in both the adjuvant and palliative setting is affected by the YAP1 activation status. Second, the relationship between the YAP1 signature and other clinical and genetic characteristics must be evaluated further. For example, IYCC patients in cohort 6 had a higher ACVR2A mutation rate than did AYCC patients. Whether the differences in frequency of this genetic event were real differences or resulted from random chance is unclear. The functional role of ACVR2A mutation in colorectal cancer cells is not well known. Therefore, more studies are warranted to determine the influence of YAP1 activation on the clinical characteristics of and genetic changes in patients with colorectal cancer. Third, all of data in current study were generated by using microarray technology and frozen tissues that are not routinely available through general practice in clinics. Therefore, it will be necessary to identify small number of robust genes (5–10 genes) well reflecting YAP1 activity in colorectal cancer and use simpler but more robust methods like qRT-PCR to measure expression of these genes with RNA from formalin-fixed paraffin-embedded tissues in future studies. Fourth, our approach, gene expression data from tumor mass only provide very limited information on tumor heterogeneity that is very critical for understanding the mechanisms of resistant to therapeutic treatment (40). Therefore, in-depth analysis of cancer cells in tumor mass by using single cell genomic approaches will be necessary to address this issues in future studies. Additional minor limitation in our prediction model includes difference in number of probes in different platforms of microarrays. However, we found no correlation between fraction of YAP1 active patients and number of probes used in prediction model.

In conclusion, our results suggest that the YAP1 signature is helpful in identifying patients with colorectal cancer with poor prognoses and/or cetuximab resistance. This signature can be further developed for the tailored management of colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K.-W. Lee, S.S. Lee, J.-S. Lee
Development of methodology: S.S. Lee, S.S. Kim, J.-S. Lee
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.-W. Lee, H.-S. Lee
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.-W. Lee, S.-B. Kim, B.H. Sohn, Y.-Y. Park, S. Kopetz, J.-S. Lee
Writing, review, and/or revision of the manuscript: K.-W. Lee, S.S. Lee, H.-S. Lee, S. Kopetz, S.C. Oh, J.-S. Lee
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.-W. Lee, H.-S. Lee, H.-J. Jang, J.-S. Lee
Study supervision: H.-S. Lee, H.-J. Jang, J.-S. Lee

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