Published OnlineFirst March 13, 2018; DOI: 10.1158/1078-0432.CCR-17-2940

Precision Medicine and Imaging

Clinical Cancer Research

TFAP2E Methylation and Expression Status Does Not Predict Response to 5-FU-based Chemotherapy in Colorectal Cancer

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Abstract

Purpose: A recent study reported that 5-fluorouracil (5-FU)-based chemotherapy is less effective in treating patients with advanced colorectal cancer demonstrating hypermethylation of the *TFAP2E* gene. The aim of our study was to confirm and validate these findings in large, uniformly treated, well-characterized patient cohorts.

Experimental Design: Two cohorts of 783 patients with colorectal cancer: 532 from a population-based, multicenter cohort (EPICOLON I) and 251 patients from a clinic-based trial were used to study the effectiveness of *TFAP2E* methylation and expression as a predictor of response of colorectal cancer patients to 5-FU–based chemotherapy. DNA methylation status of the *TFAP2E* gene in patients with colorectal cancer was assessed by quantitative bisulfite pyrosequencing analysis. IHC analysis of the TFAP2E protein expression was also performed.

Results: Correlation between TFAP2E methylation status and IHC staining was performed in 607 colorectal cancer samples. Among 357 hypermethylated tumors, only 141 (39.6%) exhibited loss of protein expression. Survival was not affected by *TFAP2E* hypermethylation in stage IV patients [HR, 1.21; 95% confidence interval (CI), 0.79–1.87; log-rank $P = 0.6$]. In stage II–III cases, disease-free survival was not influenced by TFAP2E hypermethylation status in 5-FU–treated (HR, 0.91; 95% CI, 0.52–1.59; log-rank $P = 0.9$) as well as in nontreated patients (HR, 0.88; 95% CI, 0.5–1.54; log-rank $P = 0.7$).

Conclusions: *TFAP2E* hypermethylation does not correlate with loss of its protein expression. Our large, systematic, and comprehensive study indicates that *TFAP2E* methylation and expression may not play a major role in predicting response to 5-FU–based chemotherapy in patients with colorectal cancer. *Clin Cancer Res; 24(12); 2820–7. ©2018 AACR.*

Introduction

The *TFAP2E* (transcription factor AP-2 epsilon) gene methylation was reported as a potential marker of responsiveness to 5-fluorouracil (5-FU)-based chemotherapy in colorectal cancer patients by Ebert and colleagues (1), who suggested the lack of response to 5-FU is probably mediated by *DKK4*, a downstream effector of the *TFAP2E* gene implicated in chemoresistance to 5-FU in colorectal cancer cell lines (2, 3). Although evidence was presented, this study had several important limitations that warrant further evaluation before consideration of the clinical usefulness of this marker. Primarily, this study interrogated a relatively small cohort of patients with colorectal cancers ($n = 220$), which was actually a combined collection of patients with advanced colorectal cancer from four different prospective trials that were analyzed together as one large cohort. Second, only a very small subset of the entire cohort was analyzed for methylation and expression status of the *TFAP2E* gene, as well as expression of the DKK4 protein. Third, the treatment regimen in this cohort was quite heterogeneous; some patients received 5-FU–based chemotherapy, others received antibody-based monotargeted therapy, while others underwent radiotherapy, and a subset of these received combined chemoradiation therapy. Thus, to truly appreciate whether *TFAP2E* methylation status could be a clinically relevant epigenetic marker for responsiveness

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-17-2940

Published OnlineFirst March 13, 2018; DOI: 10.1158/1078-0432.CCR-17-2940

to 5-FU in colorectal cancer, we believe that external validation is important before an extended use into routine clinical practice (4). On the basis of Ebert and colleagues' results, we evaluated the overall survival and disease-free survival in patients with colorectal cancer treated with 5-FU–based chemotherapy from two large, uniformly treated, well-characterized colorectal cancer patient cohorts, in relation to their *TFAP2E* methylation status.

Materials and Methods

Patients

This retrospective, analytic observational study included a total of 783 patients with colorectal cancer that were enrolled as part of two different groups. In the first group, 532 stage II–IV patients were enrolled as part of the population-based, EPICOLON-I project, between the years 2000 and 2001, where patients received primarily 5-FU–based chemotherapy according to clinical criteria following standard schedules and doses (5–8). The vast majority of patients in this cohort received 5-FU + leucovorin, and only 9% received FOLFOX (5-FU + oxaliplatin) or FOLFIRI (5-FU + irinotecan) regimens. In stage IV patients, 79% received 5-FU + leucovorin as first line, and 21% received FOLFOX or FOLFIRI. In the second group, 251 patients enrolled from a clinic-based trial where all patients with nonmetastatic disease received 5-FU–based adjuvant chemotherapy, and patients with stage IV colorectal cancer received the FOLFOX regimen (9). The patients included in this study were enrolled between 1996 and 2008. All stage II and III patients were treated with 5-FU–based adjuvant chemotherapy for 6 months subsequent tumor resection, and all stage IV patients were treated with 5-FU and oxaliplatin until the treatment failed. The clinicopathologic and molecular features of patients are described in Supplementary Tables S1 and S2. A flow diagram of the patients included in the study can be seen in Fig. 1. The study was approved by the institutional ethics committee of each participating hospital, and written informed consent was obtained from all patients.

Specimen characteristics, DNA extraction, and bisulfite modification

DNA from formalin-fixed, paraffin-embedded material (colorectal tumors, normal colorectal tissue) was extracted using the QIAamp DNA Mini Kit and the QIAcube (Qiagen), according to the manufacturer's protocol. Genomic DNA was modified with sodium-bisulfite using the EZ Methylation Gold Kit (Zymo Research) prior to PCR amplification for determination of the methylation status of the *TFAP2E* gene.

TFAP2E methylation status

DNA methylation status of the *TFAP2E* gene in patients with colorectal cancer was assessed by quantitative bisulfite pyrosequencing analysis using a PSQ HS 96A pyrosequencing system (Qiagen) on a bisulfate-modified genomic DNA template (10, 11). We designed two different pyrosequencing assays that encompassed both CpG islands mapped to the *TFAP2E* gene; the first was located within its promotor region/exon 1, and the second was located within intron 3, both reported by Ebert and colleagues (ref. 1; Supplementary Fig. S1). As specified in Supplementary Data, we calculated the threshold to distinguish methylated versus nonmethylated samples using matched tissues from tumor (C) and adjacent mucosa (NC) and a within-subject. ROC analysis was performed. Primer sequences used for the methylation studies can be seen in Supplementary Table S3.

IHC of TFAP2E protein expression

IHC analysis of the TFAP2E protein expression was performed only in the EPICOLON cohort, using the staining protocol and polyclonal anti-TFAP2E antibody generously provided by Dr. C. Rocken, Department of Gastroenterology, Hepatology and Infectious Diseases, Otto-von-Guericke University, Magdeburg, Germany (1). Staining was evaluated and scored by two expert pathologists (C. Egoavil and C. Alenda), who were blinded to the results of *TFAP2E* methylation for the samples. A tumor was considered to have normal expression for TFAP2E when unequivocal nuclear staining was observed in the neoplastic epithelial cells, while samples were not scored when no staining of internal control was visible. A tumor was considered to have normal expression for TFAP2E when unequivocal nuclear staining was seen in some neoplastic epithelial cells, with or without cytoplasmic staining. When the staining intensity was strong and homogeneous at 10×, the case was scored as 3; if the staining was strong but heterogeneous at 20×, it was scored as 2, and the patient was considered to have a score of 1 when the staining was light and heterogeneous at 40× (Fig. 2). Samples were not scored when no staining of internal control was visible. Tumor cells were judged as negative for protein expression only if they lacked nuclear staining in a sample in which stroma cells were stained. (Fig. 2).

Statistical analysis

Continuous variables are reported as mean ± SD, while categorical variables are reported as frequency or percentages. The determination of the cut-off value of methylation was performed

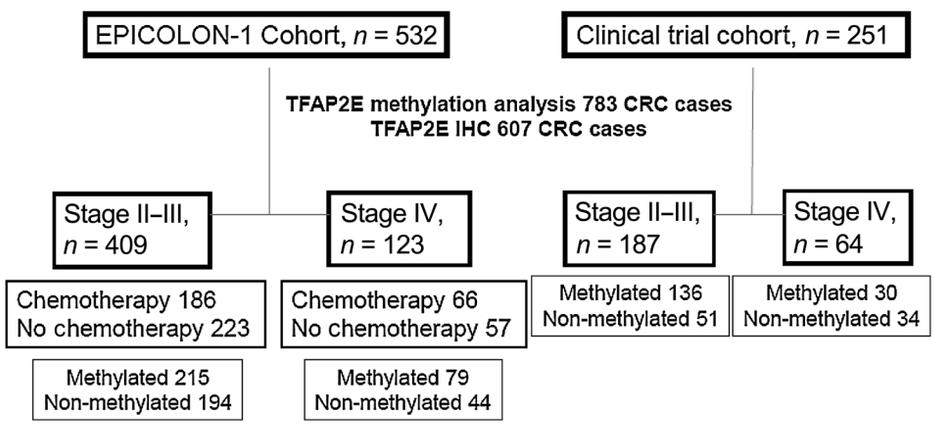

Figure 1.
Flow diagram of the participants in the study.

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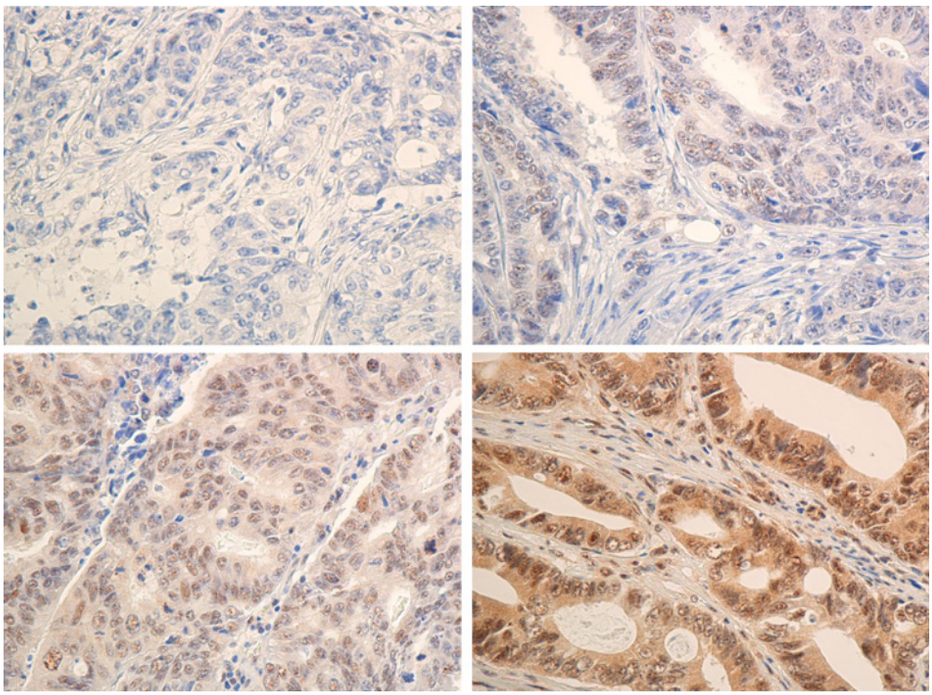

Figure 2.
IHC evaluation of TFAP2E expression. IHC staining with a polyclonal anti-TFAP2E antibody was broken down into two categories in colorectal cancer epithelial cells: positive (**B**–**D**) and negative (**A**) TFAP2E expression. Positive samples were estimated on scales of 1 to 3 depending of the intensity of tumor cells staining on each slide. **B,** Score 1 for colorectal cancer epithelial cells with weak and heterogeneous positive TFAP2E expression. Score 2 for colorectal cancer epithelial cells with strong and heterozygous positive TFAP2E expression (**C**) and score 3 (**D**) colorectal cancer epithelial cells with strong and homozygous positive TFAP2E expression. Left, 40× magnification, right, 20× magnification.

using within-subject ROC analysis. Differences in the probability of overall survival (OS) or disease-free survival (DFS) were analyzed using the v2 test. Survival curves were generated according to the Kaplan–Meier method, and univariate survival distributions were compared using a log-rank test. A multivariate analysis for determining the hazard risk ratios for death or tumor recurrence was performed using Cox proportional hazards regression analysis. All reported *P* values are two-sided, and *P* values less than 0.05 were considered to be significant.

Results

TFAP2E methylation at the two TFAP2E CpG islands

Methylation analysis of *TFAP2E* gene was performed in 783 cases. We found 58.7% (460) colorectal cancers with TFAP2E hypermethylated. TFAP2E methylation was predominantly found in CpG-island2 (intron 3) and very rarely in CpG-island1 (promoter/Exon) (Supplementary Fig. S2A). Methylation levels were significantly higher in tumor tissues (C) compared with adjacent normal mucosa (NC) in CpG-island2 within the intron 3 region of the *TFAP2E* gene ($P < 0.001$; Supplementary Fig. S2B–S2C); however, no differences were observed within the promoter region. We determined the *TFAP2E* methylation cut-off threshold at 40% that could distinguish methylated versus nonmethylated samples in the intron 3 region using a quantitative pyrosequencing assay (Supplementary Fig. S2D).

TFAP2E gene methylation status and its correlation with TFAP2E protein expression

TFAP2E protein expression was determined by IHC from all paraffin-embedded colorectal cancer tissues from EPICOLON-I patients (660; Fig. 2). A total of 607 samples were successfully scored for TFAP2E staining, while 53 cases had no internal control stain and were excluded from further analysis. Based upon IHC analysis, 65.8% (399/607) patients with colorectal cancer were classified as TFAP2E-positive.

Correlation between TFAP2E methylation status and IHC staining was performed in these 607 colorectal cancer samples. Only 141 (39.6%) from 357 hypermethylated tumors exhibited loss protein expression. It should be noted that 184 tumors from 250 tumors TFAP2E hypomethylated, retained TFAP2E immunoexpression (73.6%); therefore, no correlation between TFAP2E methylation and IHC expression was found. These results suggest that TFAP2E hypermethylation is a stochastic event and have no bearing on regulation of its expression (Supplementary Fig. S3A–S3D).

Influence of *TFAP2E* methylation on prognosis and chemotherapeutic response in patients with metastatic colorectal cancer from the EPICOLON-I cohort

From the EPICOLON-I cohort, 123 patients with metastatic colorectal cancer (mCRC) were available with a median follow-up of 518 days (1.4 years; range, 0–2148 days), 64.2% (79/123) patients had *TFAP2E* hypermethylated tumors, and there were no differences in overall survival (OS) according to *TFAP2E* hypermethylation status, (HR, 1.21; 95% CI, 0.79–1.87; log-rank $P = 0.6$; Fig. 3A). Within this subset of patients, 53.7% (66/123) received chemotherapy treatment, and 65.2% (43/66) received 5-FU–based chemotherapy. Patients who received 5-FU–based chemotherapy had similar OS independently of their *TFAP2E* hypermethylation status (methylated: 69.7%, nonmethylated: 30.3%; HR, 0.677; 95% CI, 0.28–1.46; log rank $P = 0.3$; Fig. 3B). Likewise, in patients who did not receive chemotherapy ($n = 57$), the OS was not affected by *TFAP2E* hypermethylation status (methylated: 60%, nonmethylated: 40%; HR, 1.4; 95% CI, 0.78–2.71; log-rank $P = 0.2$; Fig. 3C).

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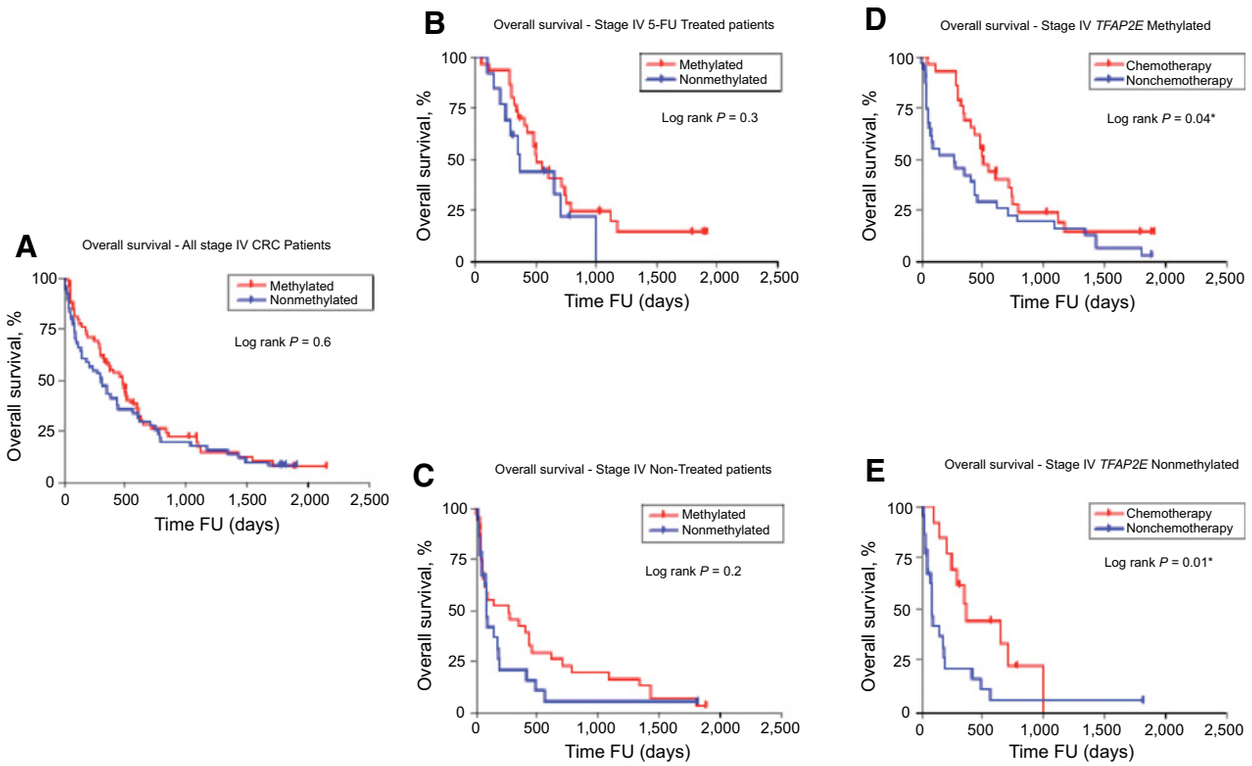

Figure 3.

Epicolon I. Overall survival of patients with stage IV colorectal cancer. *TFAP2E* methylation. **A,** Overall survival of patients with stage IV disease during FU (follow-up), according to *TFAP2E* methylation status. Overall survival of patients that received (**B**) or did not receive (**C**) chemotherapy according to *TFAP2E* methylation status. Overall survival of patients with stage IV disease and *TFAP2E* methylated tumors (**D**) and *TFAP2E* nonmethylated tumors (**E**).

Furthermore, when we analyzed the 5-FU chemotherapy effect on OS by *TFAP2E* methylation status in patients with mCRC, we realize that OS improved in both groups; patients with *TFAP2E*-methylated tumors (chemotherapy: 47.6%, nonchemotherapy: 52.4%; HR, 0.58; 95% CI, 0.33–0.99; log-rank $P = 0.04$; Fig. 3D) and *TFAP2E* nonmethylated tumors (chemotherapy: 37.1%, nonchemotherapy: 62.9%; HR, 0.39; 95% CI, 0.17–0.79; log-rank $P = 0.01$; Fig. 3E). Thus, patients with advanced colorectal cancer significantly benefit from 5-FU chemotherapy treatment independently of *TFAP2E* methylation status.

In the same way, there were no differences in OS regarding TFAP2E IHC expression status (HR, 0.84; 95% CI, 0.47–1.48; log-rank $P = 0.6$) between patients with mCRC. (Supplementary Fig. S4A).These lack of association was similar in treated (HR, 0.62; 95% CI, 0.29–1.35; log-rank $P = 0.2$; Supplementary Fig. S4B) or nontreated patients (HR, 1.1; 95% CI, 0.45–2.72; log-rank $P = 0.8$; Supplementary Fig. S4C).

Influence of *TFAP2E* methylation on prognosis and treatment response in patients with nonmetastatic colorectal cancer in the EPICOLON-I cohort

A total of 409 patients from the EPICOLON-I cohort, stage II-III were analyzed (55.5% stage II). Median follow-up of these patients was 1187 days (3.2 years; range, 0–2184 days). Of the 409 patients, 215 (52.6%) had *TFAP2E* hypermethylated tumor. At the end of the follow-up period, 36.2% (148/409) patients died, and the median follow-up for this group was 703 ± 518 days (1.9 ± 1.4 years). Tumor recurrence following surgery was seen in 29.9% (120/409) patients, with a median recurrence time of $1{,}015 \pm 559$ days (2.9 ± 1.5 years). Adjuvant chemotherapy was given to 45.5% (186/409) patients, which included 177 who received 5-FU + leucovorin.

We analyzed the effect on disease-free survival (DFS) of TFAP2E methylation status in patients with stage II–III colorectal cancer (5-FU–treated and nontreated). There were no differences in DFS of 5-FU–treated (methylated: 51.4%, nonmethylated: 48.6%; HR, 0.91; 95% CI, 0.52–1.59; log-rank $P = 0.9$; Fig. 4A) or nontreated patients (methylated: 56.4%, nonmethylated: 43.6%; HR, 0.88; 95% CI, 0.5–1.54; log-rank $P = 0.7$; Fig. 4B). This lack of difference remained unchanged when we analyzed separately stage II (227 patients, 119, 51.5% methylated; 102, 48.5% nonmethylated; log-rank $P = 0.7$) and stage III (182 patients, 87, 47.8% methylated; 95, 52.2% nonmethylated) patients (log-rank $P = 0.9$).

At the same time, we found that 5-FU–based chemotherapy did not improve DFS in stage II–III patients, independently of TFAP2E methylation tumors' status (TFAP2E methylated: chemotherapy: 51.4%, nonchemotherapy: 48.6%; HR, 0.78; 95% CI, 0.45–1.35; log-rank $P = 0.4$, Fig. 3C; and TFAP2E nonmethylated: chemotherapy: 52.1%, nonchemotherapy: 47.9%; HR, 0.79; 95% CI, 0.44–1.41; log-rank $P = 0.4$; Fig. 4D).

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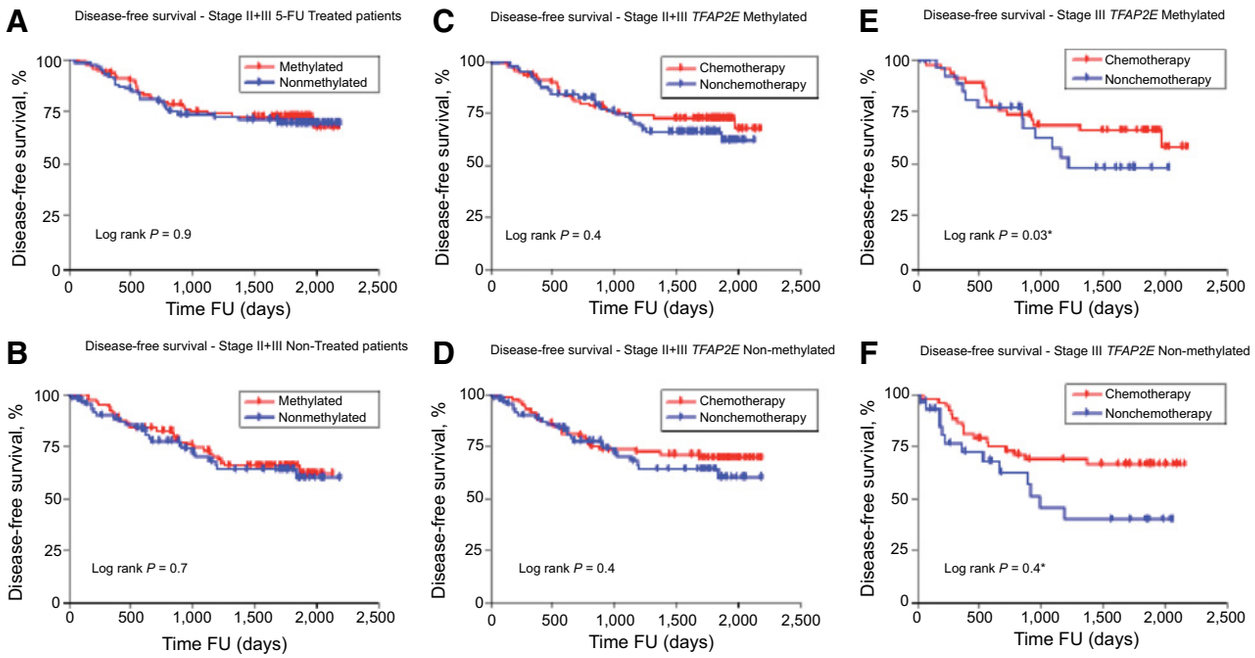

Figure 4.

EPICOLON I. Disease-free survival stage II and III colorectal cancer. *TFAP2E* methylation. Disease-free survival of patients with stage II and III disease during FU (follow-up) that received (**A**) or did not receive (**B**) adjuvant chemotherapy according to *TFAP2E* methylation status. **C,** Disease-free survival of patients with stage II+III colorectal cancer and *TFAP2E* methylated and (**D**) *TFAP2E* nonmethylated tumors (**E**) disease-free survival of patients with stage III colorectal cancer and *TFAP2E* methylated and (**F**) *TFAP2E* nonmethylated tumors.

In addition, chemotherapy with 5-FU only in patients with stage III colorectal cancer, improved DFS in both, patients with TFAP2E methylated tumors (chemotherapy: 46.6%, nonchemotherapy: 53.4%; HR, 0.57; 95% CI, 0.31–0.96; log-rank $P = 0.03$, Fig. 4E) and TFAP2E nonmethylated tumors (chemotherapy: 54.4%, nonchemotherapy: 45.6%; HR, 0.51; 95% CI, 0.20–0.99; log-rank $P = 0.04$; Fig. 4F).

Similarly, when we analyzed the effect of TFAP2E IHC expression in patients with stage II–III colorectal cancer, we found that DFS was not affected (HR, 0.83; 95% CI, 0.52–1.35; log-rank 0.5; Supplementary Fig. S4D) by low or high expression, and also there were no differences regarding TFAP2E expression in 5-FU–treated (HR, 0.8; 95% CI, 0.33–1.3; log-rank 0.6; Supplementary Fig. S4E) or nontreated patients (HR, 0.63; 95% CI, 0.33–1.29; log-rank 0.2; Supplementary Fig. S4F).

Influence of *TFAP2E* methylation on survival in clinical cohort of colorectal cancers

A total of 64 patients with mCRC were included from 251 clinic-based cohorts. All subjects received FOLFOX (5-FU + oxaliplatin), and the median follow-up was 734 days (2 years; range, 0–2,511 days). A total of 46.9% (30/64) patients had *TFAP2E* hypermethylated tumors, and the OS was not affected by *TFAP2E* methylated status (methylated: 46.9%, nonmethylated: 53.1%; HR, 0.89; 95% CI, 0.52–1.51; log-rank $P = 0.7$; Fig. 5A).

A total of 187 stage II and III patients from this cohort were analyzed (38.5% stage II). All patients underwent 5-FU + leucovorin adjuvant chemotherapy after surgery. There were 40 (21.4%) deaths over the mean follow-up time of 1,028 ± 867days (2.8 ± 2.3 years), 33 patients died due to cancer-related causes, while seven patients had other causes of death. Tumor recurrence was seen in 36.4% (68/187) patients, at a median time of 801 ± 723 days (2.1 ± 1.8 years) after surgery.

In this cohort, a total of 136 patients (72.7%) showed TFAP2E hypermethylation. Patients treated with 5-FU–containing adjuvant chemotherapy who had *TFAP2E* hypermethylation tumors showed worse outcome than patients with nonmethylated tumors (log-rank $P = 0.03$, Fig. 5B). This trend was maintained in the subgroup analysis for stage II patients (log-rank $P = 0.01$, Fig. 5C), but not for stage III patients (log-rank $P = 0.2$; Fig. 5D).

In the multivariable Cox regression analysis, *TFAP2E* methylation was an independent predictor of early recurrence for stage II–III and stage II colorectal cancer patients, respectively (stage II+III: HR, 1.91; 95% CI, 1.02–3.58; $P = 0.045$; stage II: HR, 1.91; 95% CI, 1.2–25.95, $P = 0.029$; Table 1), hence suggesting a prognostic value from *TFAP2E* methylation in patients with curative colorectal cancer who received uniform 5-FU adjuvant treatment.

Discussion

This study was designed to evaluate the role of *TFAP2E* methylation, a novel biomarker previously reported (1), as a predictive factor of therapeutic response and prognosis to 5-FU–based chemotherapy in patients with colorectal cancer. Ebert and colleagues had presented evidence that 5-FU–based chemotherapy was ineffective in patients with colorectal cancer with *TFAP2E* hypermethylation; however, this is based on the analysis of a

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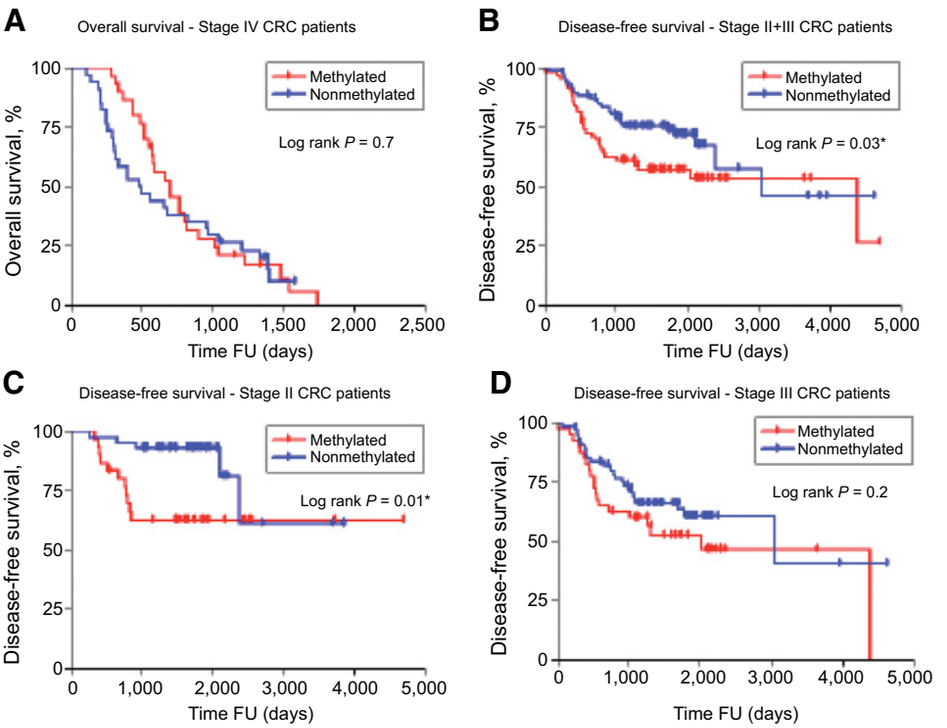

Figure 5.
Clinical cohort. Overall survival stage of patients with stage IV colorectal cancer. Disease-free survival of patients with stages II and III colorectal cancer. *TFAP2E* methylation. **A,** Overall survival of patients with stage IV colorectal cancer during FU (follow-up), according to *TFAP2E* methylation status. (**B**) Disease-free survival of patients with stage II+III colorectal cancer, according to *TFAP2E* methylation status (**C**). Disease-free survival of patients with stage II colorectal cancer, according to *TFAP2E* methylation status (**D**). Disease-free survival of patients with stage III colorectal cancer, according to *TFAP2E* methylation status.

relatively small, heterogeneous subset of patients with advanced colorectal cancer who were treated with nonuniform chemotherapeutic regimens and, as we know there is no validation reports of these findings in a larger sample group. This step is crucial before testing this biomarker in an appropriate prospective trial and implementing its use in molecular diagnostic laboratories. Therefore, we used two large and well-characterized cohorts of patients with colorectal cancer: one a population-based study and the other a clinic-based trial. Both cohorts of patients with colorectal cancer were uniformly treated with 5-FU–based chemotherapeutic regimens. As a result, our large, systematic and comprehensive analysis shows two things. First, *TFAP2*E hypermethylation does not correlate with loss of TFAP2E protein expression, and second, neither *TFAP2E* methylation nor *TFAP2E* expression predict response to 5-FU–based adjuvant chemotherapy.

Previous analysis regarding this biomarker was conducted in a small group of patients with colorectal cancer ($N = 28$), and this inverse correlation between methylation and expression levels did not reach statistical significance (1); however, they concluded that hypermethylation of the *TFAP2E* gene conveys suppression of TFAP2E protein expression. In contrast, following a systematic evaluation of the relationship between *TFAP2E* hypermethylation and TFAP2E protein expression in our population-based cohort, we unequivocally demonstrated that although the intron-3 CpG island within the *TFAP2E* gene is heavily methylated in tumor samples compared to normal mucosa, this epigenetic alteration

Table 1. Multivariate analysis of disease-free survival in patients with stage II and III colorectal cancer from the clinical cohort

Stage II + III	**Univariate**		**Multivariate**	
Covariate	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
*Age (>median (66) vs. <median)	1.02 (0.65–1.61)	0.93	—	—
Gender (male vs. female)	1.02 (0.64–1.62)	0.94	—	—
*Tumor size (>45 mm vs. <45 mm)	1.08 (0.66–1.76)	0.76	—	—
Vascular invasion (positive vs. negative)	1.29 (0.74–2.49)	0.47	—	—
Mucinous (positive vs. negative)	1.36 (0.64–2.62)	0.32	—	—
Perineural invasion (positive vs. negative)	1.87 (1.01–3.45)	0.046	1.73 (0.89–3.34)	0.11
Lymph node (positive vs. negative)	**1.89 (1.14–3.12)**	**0.014**	**2.17 (1.06–4.46)**	**0.034**
TFAP2E methylation (high vs. low)	**1.81 (1.10–2.98)**	**0.019**	**1.91 (1.02–3.58)**	**0.045**
Stage II				
Covariate				
*Age (>median (66) vs. <median)	0.66 (0.27–1.63)	0.37		
Gender (male vs. female)	0.76 (0.32–1.78)	0.53	—	—
*Tumor size (>45 mm vs. <45 mm)	0.89 (0.35–2.25)	0.79	—	—
Mucinous (positive vs. negative)	0.8 (0.18–3.47)	0.77	—	—
Perineural invasion (positive vs. negative)	1.5 (0.46–4.86)	0.49	—	—
Vascular invasion (positive vs. negative)	2.17 (0.67–7.05)	0.2	2.11 (0.59–7.51)	0.25
TFAP2E methylation (high vs. low)	**3.14 (1.09–9.01)**	**0.035**	**1.91 (1.20–25.95)**	**0.029**

Abbreviations: CI, confidence interval; *, median values used. *TFAP2E* methylation (high >40%; low ≤40%).

Published OnlineFirst March 13, 2018; DOI: 10.1158/1078-0432.CCR-17-2940

does not lead to the transcriptional suppression of TFAP2E expression in the colon. This was further highlighted by our observation for coexistence of significant hypermethylation but no loss of the corresponding protein expression for TFAP2E in our large subset of colorectal cancers. Although unclear, we hypothesized that this lack of correlation between methylation and expression might be due to the fact that gene methylation could be analyzed in CpG whitin a nonregulatory region of the gene (1). Recent evidence has shown the association of intronic methylation with alternative splicing (12, 13) and noncoding RNAs regulation; (14) however, its relationship with gene silencing is not clear (15, 16), and is often related to intron 1 (17, 18). This may be due to an extension of methylation changes in the regulatory exon 1 CpG island within genes. Other possibilities such as posttranslational changes can also explain these discrepancies between methylation and protein expression.

The most important conclusion made by Ebert and colleagues was that patients with colorectal cancer with *TFAP2E* hypermethylated tumors do not benefit from 5-FU–based chemotherapy (1). In another study performed in a cohort of patients with I–III stage colorectal cancer (Park and colleagues, Oncology 2015), an independent correlation between TFAP2E methylation and better prognosis was found, receiving or not adjuvant chemotherapy. However, our extensive validation of these results were unsuccessful in the two patient cohorts we analyzed, wherein, the presence of *TFAP2E* methylation in tumors did not have any effect on the response to 5-FU–based chemotherapy in patients with metastatic colorectal cancers. Furthermore, we found that *TFAP2E* methylation levels seems not to influence DFS in patients with stage II and III colorectal cancer from EPICOLON-I treated or no with 5-FU–based chemotherapy. The results of our validation suggest that *TFAP2E* methylation status may not be a predictive marker for response to adjuvant 5-FU–based chemotherapy in patients with colorectal cancer. However, our results do not allow us to discard a prognostic value for this marker, especially in 5-FU–treated patients with stage II colorectal cancer.

Nevertheless, it is important to point out that our study may have some limitations. In our population-based cohort, the treatment decision was not random, and chemotherapy was decided using clinical criteria. In the clinical cohort, there was no group of nontreated patients. Moreover, follow-up duration was not very long, and possibly some recurrences can be missed. However, these limitations aside, given the strength of our large, well-characterized group of patients with colorectal cancer, we believe that our interpretation is a reliable reflection of the role of *TFAP2E* in colorectal cancer.

In summary, in this study of the role methylation and expression of the *TFAP2E* gene may play in the response to 5-FU–based chemotherapy, we demonstrated that although methylation in the *TFAP2E* intron 3 is tumor-related, it does not correlate with loss of its protein expression, and more importantly, *TFAP2E* methylation does not play any role in predicting response to 5-FU–based chemotherapy in patients with colorectal cancer. Interestingly, we did make the observation that *TFAP2E* methylation was an independent predictor of early recurrence, especially for patients with stage II colorectal cancer in the clinical cohort of patients. Further appropriate retrospective or prospective clinical trials are required to confirm these results in future.

Disclosure of Potential Conflicts of Interest

C.R. Boland has received speakers bureau honoraria from Ambry Genetics. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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Acknowledgments

This work was supported by the grants CA72851, CA181572, CA184792, CA187956, and CA202797 from the National Cancer Institute, NIH, a grant (RP140784) from the Cancer Prevention Research Institute of Texas (CPRIT), pilot grants from the Baylor Sammons Cancer Center and Foundation, as well as funds from the Baylor Research Institute. This work was also supported by Instituto de Salud Carlos III (PI08/0726, PI11/2630 INT-12-078, INT13-196, PI14/01386). L. Perez-Carbonell is a recipient of a post-doctoral grant from Fundación Alfonso Martín Escudero 2012.

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Received October 9, 2017; revised January 17, 2018; accepted March 1, 2018; published first March 13, 2018.

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Published OnlineFirst March 13, 2018; DOI: 10.1158/1078-0432.CCR-17-2940

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Clinical Cancer Research

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Clin Cancer Res 2018;24:2820-2827. Published OnlineFirst March 13, 2018.