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Oncogenes and Tumor Suppressors

Molecular Cancer Research

AKT1 E17K in Colorectal Carcinoma Is Associated with BRAF V600E but Not MSI-H Status: A Clinicopathologic Comparison to PIK3CA Helical and Kinase Domain Mutants

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Abstract

The PI3K/AKT/mTOR pathway is activated through multiple mechanisms in colorectal carcinoma. Here, the clinicopathologic and molecular features of *AKT1* E17K–mutated colorectal carcinoma in comparison with PIK3CA-mutated colorectal carcinoma are described in detail. Interestingly, in comparison with *PIK3CA* mutants, *AKT1* E17K was significantly associated with mucinous morphology and concurrent *BRAF* V600E mutation. Among *PIK3CA* mutants, exon 21 mutations were significantly associated with *BRAF* V600E mutation, MSI-H status, and poor differentiation, while exon 10 mutations were associated with *KRAS*/*NRAS* mutations. Three of four *AKT1* mutants with data from both primary and metastatic lesions had concordant *AKT1* mutation status in both. Both *AKT1*- and *PIK3CA*-mutant colorectal carcinoma demonstrated frequent loss of PTEN expression (38% and 34%, respectively) and similar rates of p-PRAS 40 expression (63% and 50%, respectively). Both patients with *AKT1* E17K alone had primary resistance to cetuximab, whereas 7 of 8 patients with *PIK3CA* mutation alone experienced tumor shrinkage or stability with anti-EGFR therapy. These results demonstrate that *AKT1* E17K mutation in advanced colorectal carcinoma is associated with mucinous morphology, *PIK3CA* wild-type status, and concurrent RAS/RAF mutations with similar pattern to *PIK3CA* exon 21 mutants. Thus, *AKT1* E17K mutations contribute to primary resistance to cetuximab and serve as an actionable alteration.

Implications: This first systematic study of *AKT1* and *PIK3CA* hotspot mutations and their association with cetuximab resistance and BRAF V600E mutation has important ramifications for the development of personalized medicine, particularly in identifying patient candidates for PI3K or AKT inhibitors. *Mol Cancer Res; 13(6); 1003–8. ©2015 AACR.*

Introduction

The phosphoinositide 3-kinase/v-akt murine thymoma viral oncogene/mammalian target of the rapamycin (PI3K/AKT/mTOR) pathway is activated in colorectal carcinoma through various mechanisms, including missense mutations in *PIK3CA* and *AKT1* (v-akt murine thymoma viral oncogene homologue 1) and loss of PTEN expression. The *PIK3CA* gene encodes the p110α catalytic subunit of PI3K. Its activation promotes carcinogenesis through increased cell proliferation and survival (1). Eighty percent of oncogenic mutations in *PIK3CA* are clustered at hotspots in exon 10 at codons 542 and 545 and exon 21 at codon 1047 (2). Recently, proximal location and mucinous histology have been associated with *PIK3CA*-mutant colorectal carcinoma (3). Differences between the molecular profiles of *PIK3CA* exon 10– and 21–mutant colorectal carcinoma include a higher prevalence of concurrent *KRAS* mutation with *PIK3CA* exon 10 mutation and higher prevalence of *BRAF* co-mutation and microsatellite instability (MSI) with *PIK3CA* exon 21 mutation (4).

In 2007, Carpten and colleagues (5) first described the pleckstrin homology domain missense mutation *AKT1* E17K in various types of human and mouse tumors, including breast, colorectal, ovarian, and myeloid malignancies. Mutant Akt (E17K) is more readily ubiquitinated and phosphorylated than wild-type Akt. The ubiquitinated-phosphorylated-Akt (E17K) translocates more efficiently to the nucleus than wild-type Akt, which may contribute to the transforming potential of E17K-Akt6 (6). Kumar and Purohit (7) have shown that the oncogenic effects of *AKT1* E17K mutation may be due to rapid conformational changes affecting activation status. Activation of the protein encoded by the gene occurs via membrane localization, which is mediated by the pleckstrin homology domain, and followed by the subsequent phosphorylation of AKT1 at S473 and T308 positions (8).

Unlike *PIK3CA* and *PTEN* alterations, *AKT1* mutations have not been thoroughly studied in colorectal carcinoma due to low numbers, although past studies have identified rare cases. While previous studies have identified rare cases, analysis of characteristics, including mutation prevalence, histopathologic features, concurrent driver mutations, and MSI status, has not been performed. In this study, we aimed to characterize the clinicopathologic, molecular, and pathway activation status of the *AKT1* pleckstrin homology domain hotspot mutation E17K and to

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compare these cases with *PIK3CA* mutants in a large cohort of colorectal carcinoma.

Patients and Methods

Patients

After approval from the local Institutional Review Board, data were reviewed for a total of 2,631 colorectal carcinoma cases consecutively submitted for clinical molecular testing at MSKCC from 2009 to 2013. Testing was primarily to assess eligibility for anti-EGFR antibody therapy. Therefore, the vast majority of cases had distant metastases. An additional 491 cases with mutational analysis were identified through the cBio portal from The Cancer Genome Atlas (TCGA; ref. 9). All 141 colorectal carcinoma cases with *AKT1* p. E17K, *PIK3CA* p.R88Q, p.E542K, p.E545K/G/D, or *PIK3CA* p. H1047L/R were included for further histopathologic, molecular (including *RAS*/*RAF* status), IHC, and clinical review.

Histopathology review

World Health Organization definitions were used to classify histopathology: mucinous histology was defined as >50% of tumor volume is composed of extracellular mucin. Moderately differentiated colorectal carcinoma was defined as 50% to 95% of tumor volume comprised of glands. Poorly differentiated colorectal carcinoma was defined as <50% of tumor volume forming glands (10).

Mutation detection

Genomic DNA was extracted from formalin-fixed paraffin-embedded tumor tissue after macrodissection using the DNeasy Tissue kit (Qiagen), following the manufacturer's standard protocol. All cases were analyzed with the MassARRAY system (Sequenom) with primers as previously described (11). Missense mutations in hotspots, including KRAS c.34, 35, 37 38, 181, 182, 183, 351, and 437; NRAS c. 34, 35, 37, 38, 181, 182, and 183; BRAF c. 1781, 1798, and 1799; PIK3CA c. 263, 1624, 1633, and 3140; and AKT1 c.49 were recorded.

MSI assays and mismatch repair protein IHC

For colorectal carcinomas harboring *AKT1* E17K, *PIK3CA* E542K, *PIK3CA* E545K/G/D, and *PIK3CA* H1047R/L/Y mutations, MSI was assessed either by IHC for mismatch repair proteins MLH1, PMS2, MSH2, and MSH6, as previously described (12), at the time of surgery or by PCR using previously extracted DNA, permitting sufficient remaining DNA. MSI analysis was performed using the MSI analysis system (Promega) according to the manufacturer's instructions. Briefly, fluorescently labeled primers for BAT-25, BAT-26, NR-21, NR-24, and MONO-27 were used for DNA amplification. The PCR products were then separated by capillary electrophoresis. The presence of additional peaks forming a second Gaussian distribution in $\geq$2 of the 5 loci was interpreted as MSI-high (MSI-H).

PTEN and p-PRAS40 IHC

IHC staining was performed for PTEN (1:100; Cell Signaling Technology) and phospho-PRAS40 (1:40; Cell Signaling Technology), as previously described (13), on cases with *AKT1* E17K, *PIK3CA* exon 10 mutations, or *PIK3CA* exon 21 mutations for which formalin-fixed paraffin-embedded tissue was available.

Statistical analysis

Statistical analyses were performed with a χ^2 test for 2 $\times$ 2 categorical analyses. Two-tailed *P* values between 0.01 and 0.05, and those less than 0.01, were reported separately to account for multiple hypothesis testing.

Results

Mutation frequency and pathway activation

Out of 2,631 cases of colorectal carcinoma genotyped between 2009 and 2013, 18 (0.7%) cases were positive for *AKT1* p. E17K (c. 49G>A) mutation, 9 (0.3%) cases were positive for *PIK3CA* exon 2 R88Q mutations, 82 (3.1%) cases were positive for PIK3CA exon 10 mutations, and 29 (1.1%) cases were positive for *PIK3CA* exon 21 mutations. Approximately one-third (33%–38%) and one half (47%–63%) of *AKT1* E17K, *PIK3CA* exon 10, and *PIK3CA* exon 21 mutants lost PTEN expression and were positive for p-PRAS40 expression, respectively. The clinicopathologic and molecular profiles of these cases are summarized in Table 1 and illustrated in Figs. 1 and 2. Outcomes of *KRAS*/NRAS/BRAF wild-type patients who received anti-EGFR therapy are summarized in Table 2.

Table 1. Clinicopathologic and molecular characteristics of colorectal carcinomas harboring *AKT1* or *PIK3CA* mutations

Clinicopathologic parameter	*AKT1* E17K	*PIK3CA* E542K, E545K/G/D	*PIK3CA* H1047R/L/Y	*PIK3CA* R88Q	All *PIK3CA*
Age (range, mean, median)	40–76, 57, 57	29–90, 58, 56	35–85, 62, 66	36–68, 58, 59	29–90, 59, 58
Sex (M:F)	10:8	39:43	12:17	3:6	54:66
Proximal location	10/18 (56%)	32/82 (39%)	14/29 (48%)	6/9 (67%)	52/120 (43%)
Hepatic metastases	14/18 (78%)	61/82 (74%)	10/29 (35%)[a,b]	4/9 (44%)	75/120 (63%)
Pulmonary metastases	9/18 (50%)	24/82 (29%)	5/29 (17%)[c]	0/9	29/120 (24%)[c]
Mucinous histology	7/21 (33%)	3/82 (4%)[a]	5/29 (17%)	1/9 (11%)	9/120 (8%)[a]
Poor differentiation	3/21 (14%)	10/82 (12%)	10/29 (35%)[b]	2/9 (22%)	22/120 (18%)
NRAS or *KRAS* mutation	11/21 (52%)	55/82 (67%)	13/29 (45%)[d]	3/9 (33%)	71/120 (59%)
BRAF V600E mutation	7/21 (33%)	5/82 (6%)[a]	8/29 (28%)[b]	0/9	13/120 (11%)[a]
MSI-H/MMR loss[e]	3/17 (18%)	10/62 (16%)	13/22 (59%)[b]	3/8 (38%)	26/92 (28%)
PTEN loss (IHC)	6/16 (38%)	6/17 (35%)	5/15 (33%)	—	11/32 (34%)
p-PRAS40 expression (IHC)	10/16 (63%)	8/17 (47%)	8/15 (53%)	—	16/32 (50%)

[a]$P < 0.01$, comparison with *AKT1* E17K.
[b]$P < 0.01$, comparison with *PIK3CA* E542K, E545K/G/D.
[c]$P < 0.05$, comparison with *AKT1* E17K.
[d]$P < 0.05$, comparison with *PIK3CA* E542K, E545K/G/D.
[e]The ratio of IHC to PCR testing (IHC:PCR) to assess MSI-H/MMR status for each PI3K mutation was as follows: *AKT1* E17K (4:13), *PIK3CA* E542K, E545K/G/D (26:36); and PIK3CA H1047R/L/Y (13:9).

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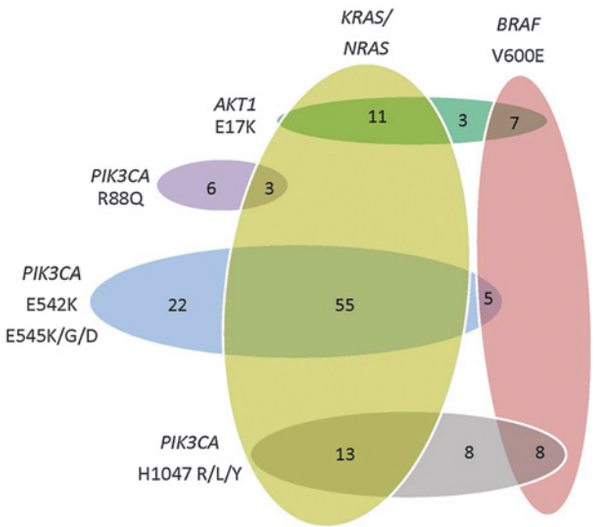

Figure 1.
Distribution of PI3K pathway mutations in colorectal carcinoma. AKT1 E17K– and PIK3CA H1047R/L/Y–mutant colorectal carcinoma had similar mutational profiles, harboring BRAF V600E co-mutations (33% and 28%, respectively) more frequently than PIK3CA E542K or E545K/G/D mutants (6%).

AKT1 E17K

All 18 institutional *AKT1* E17K cases had distant metastases. The site tested was primary tumor for 12 (67%) and distant metastasis for 6 (33%) cases. *AKT1*-mutated colorectal carcinoma was significantly associated with mucinous morphology ($P = 0.0006$) compared with *PIK3CA*-mutated colorectal carcinoma. Comparing exon-specific subsets, *AKT1* mutants, like *PIK3CA* exon 21 mutants, were more likely to have mucinous differentiation and *BRAF* V600E co-mutation than *PIK3CA* exon 10 mutants ($P = 0.0001$).

Of the 18 *AKT1* E17K cases, 15 (83.3%) cases had concurrent RAS/RAF–activating mutations in the same sample, including 6 (33.3%) *KRAS* exon 2 mutations, 5 (27.8%) *BRAF* V600E mutations, 2 (11.1%) *KRAS* exon 4 mutation, 1 (5.9%) *NRAS* exon 3 mutation, and 1 (5.9%) case with both a *KRAS* exon 2 and an *NRAS* exon 3 mutation. The mutation status of the last case was confirmed by repeat extraction and Sanger sequencing. In 4 patients, material was available to correlate the *AKT1* status of both primary and metastasis. All 4 patients had the same RAS/RAF–activating mutation in the primary and metastasis. Three of these 4 patients had *AKT1* E17K mutations in both primary and metastasis. Interestingly, the fourth patient harbored an *AKT1* E17K mutation (concurrent with *KRAS*) in a liver metastasis and a *PIK3CA* E542K mutation in the primary tumor (with the same *KRAS* mutation). These findings are suggestive of convergent, or parallel, evolution in the PI3K pathway (14). Two patients with *AKT1* E17K mutations who had wild-type *KRAS* and *BRAF* received cetuximab, and both demonstrated primary resistance.

Three additional *AKT1* E17K–mutated cases were identified via the cBioPortal of The Cancer Genome Atlas, among 491 total colorectal carcinoma. One case had mucinous features and harbored a *BRAF* V600E mutation. The other two cases were moderately differentiated, one with a *BRAF* V600E mutation and the other with a *KRAS* A146T mutation.

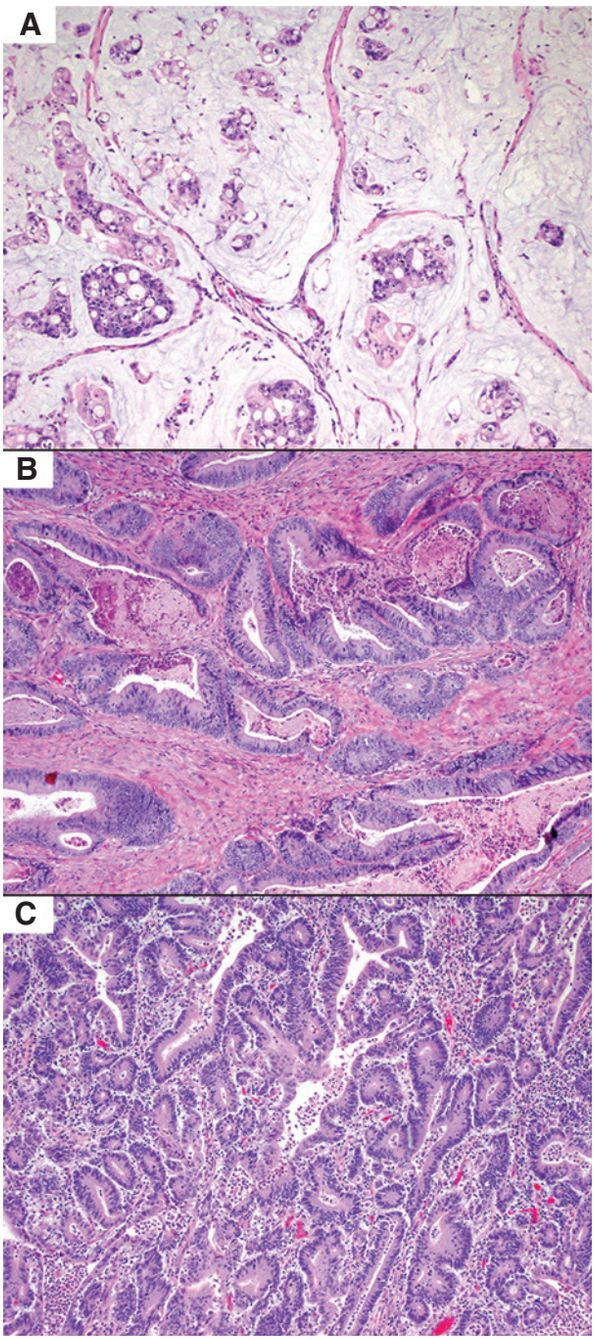

Figure 2.
Histologic features of AKT1 E17K- and PIK3CA-mutant colorectal carcinoma. A, AKT1 E17K–mutant colorectal carcinomas more frequently had mucinous histology (33% of cases), while PIK3CA E542K and E545K/G/D mutants (B) had conventional histology in 84% of cases. C, PIK3CA H1047R/L/Y–mutant colorectal carcinoma more frequently were MSI-H/mismatch repair protein deficient (59% of cases), with histologic features including increased intratumoral lymphocytes (A–C: H&E, ×100).

PIK3CA exon 10

Seventy of 82 (85.4%) *PIK3CA* exon 10 mutations, including E542K (32), E545K (44), E545G (5), and E545D (1), were identified in stage IV patients. The site tested was the primary

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Table 2. Response to anti-EGFR therapy in patients with stage IV *KRAS/BRAF* wild-type, PI3K/Akt pathway–mutant colorectal carcinomas

PI3K pathway mutation	Synchronous interventions/chemotherapies	Outcome
PIK3CA R88Q	Fluorouracil, irinotecan, floxuridine, hepatic resection	No evidence of disease for at least 24 months
PIK3CA E542K	Irinotecan	Radiologic response for at least 5 months, including disappearance of multiple 1- to 2-cm hepatic metastases with stable size of peritoneal metastases
PIK3CA E542V	None	Stable disease for 6 months
PIK3CA E542K	Irinotecan	Stable disease at 1 month, progression of disease at 3 months
PIK3CA E545K	Irinotecan, fluorouracil, leucovorin, radiofrequency ablation, hepatic resection and infusion pump	Stable disease for at least 9 months
PIK3CA E545K	Irinotecan	Stable disease for 10 months before progression
PIK3CA E545K	Irinotecan	No evidence of disease for at least 14 months (macroscopic disease resected before cetuximab)
PIK3CA H1047R	Yttrium-90	Stable disease for 6 months
AKT1 E17K	Irinotecan	Progression of disease without any period of stabilization or response
AKT1 E17K	Irinotecan	Progression of disease without any period of stabilization or response

tumor for 51 (62.2%) and distant metastasis for 31 (37.8%) cases. Primary tumors were located in the proximal colon ($n = 32$, 39.0%), transverse ($n = 8$, 9.8%), and distal colon ($n = 42$, 51.2%). In comparison with *PIK3CA* exon 21 mutants, these cases less frequently had poor differentiation ($P = 0.007$) and more frequently had hepatic metastases ($P = 0.0001$).

Sixty (72.3%) cases had activating RAS/RAF mutations detected in the same sample. Of these, 49 harbored *KRAS* exon 2 mutations, 5 harbored *BRAF* V600E mutations, 3 had *KRAS* exon 4 mutations, 1 had a *KRAS* exon 3 mutation, and 1 had mutation in both *KRAS* and *NRAS* at exon 2. Compared with exon 21 kinase domain mutations, *PIK3CA* exon 10–mutated colorectal carcinomas showed a higher prevalence of *NRAS*/*KRAS* mutations and a lower prevalence of *BRAF* mutations ($P = 0.035$ and 0.002, respectively). Twenty-three (23.7%) cases were wild-type for *KRAS*, *BRAF*, and *NRAS*.

PIK3CA exon 21

A total of 29 cases had *PIK3CA* exon 21 mutations: H1047R (25), H1047L (3), and H1047Y (1). Two cases with H1047R mutations had a second *PIK3CA* mutation: C420R in one and R88Q in the other. Twenty-two cases (75.9%) were stage IV. The site tested was the primary tumor for 23 (79.3%) and distant metastasis for 6 (20.7%) cases.

Twenty-one (72.4%) patients had RAS/RAF co-mutations. These included 12 (41.4%) *KRAS* exon 2 mutations, 8 (27.6%) with *BRAF* V600E mutations, and 1 (3.5%) *KRAS* exon 3 mutation. Eight patients were wild-type for *KRAS*, *NRAS*, and *BRAF*. Thirteen of 21 (59%) cases with available material for microsatellite instability polymerase chain reaction or mismatch repair protein immunohistochemistry were microsatellite instability-high/mismatch repair deficient ($P = 0.0002$), compared with all other *PIK3CA* mutants.

PIK3CA R88Q

The clinicopathologic features of the 9 *PIK3CA* mutation R88Q are summarized in Table 1. Five patients were stage IV. Four patients had liver metastases and 2 had intra-abdominal metastases. The site tested was primary tumor for 4 patients and metastasis for 5 patients. Only 3 patients (33%) in this group also harbored a RAS/RAF mutation. These included 2 *KRAS* exon 2 mutations and 1 *KRAS* exon 4 mutation. This contrasts with the helical and kinase domain *PIK3CA* mutants as well as the *AKT1* E17K mutants, which had RAS/RAF driver mutations in over 70% of cases.

Discussion

This study is the first to characterize the clinical, pathologic, molecular, and pathway activation properties of colorectal carcinoma harboring *AKT1* E17K mutations, with comparison to *PIK3CA* mutations. We find that colorectal carcinoma with *AKT1* E17K mutation has a higher incidence of concurrent *BRAF* V600E mutations and mucinous histology in comparison with *PIK3CA* exon 10–mutated colorectal carcinoma. Our finding that *PIK3CA* exon 21 mutations have a higher prevalence of *BRAF* mutations and MSI-H status than *PIK3CA* exon 10 mutations is in agreement with recent reports from other groups. The prevalence of mucinous histology by *PIK3CA* mutation in our cohort was also in line with previous work (4).

The *AKT1* mutations were concurrent with activating mutations in the RAS/RAF pathway in the majority (85%) of cases, but were consistently mutually exclusive with *PIK3CA* mutations. The 3 cases of *AKT1* E17K mutation that occurred in the absence of RAS/RAF mutations shared clinical features with other *AKT1*-mutant cases, including mucinous histology and microsatellite stable status.

The impact of *AKT1* mutation on response to anti-EGFR therapy has not been previously studied. Two of these 3 patients received anti-EGFR therapy (cetuximab), and both patients were resistant to this treatment. In comparison, of 8 patients with *PIK3CA*-mutant and *KRAS*/*BRAF* wild-type colorectal carcinoma, 7 (88%) exhibited either tumor regression or stability when treated with anti-EGFR therapy, either alone or in combination with other therapies (Table 2). A possible explanation for this different response to anti-EGFR therapy is that mutant PIK3CA leads to increased phosphorylation and dependence on ERK, which can be inhibited by cetuximab, while AKT1 E17K mutation does not increase phosphorylation of ERK1/2 (15). While anti-EGFR therapy may not be an effective therapeutic option for these patients, targeted therapy against the AKT/mTOR pathway may be an emerging option in patients with metastatic disease.

Similar to *PIK3CA* H1047R/L/Y–mutated colorectal carcinoma, AKT1 E17K colorectal carcinoma showed a high rate of concurrent *BRAF* V600E mutation (28% for both *PIK3CA* exon 21 and *AKT1* institutional cases), yet not as high of a rate of MSI

(59% for *PIK3CA* exon 21 vs. 18% for *AKT1*). This relative enrichment of microsatellite-stable *BRAF* V600E mutants might account for the higher number of colorectal carcinoma with distant metastases (75.9% vs. 100%) seen in our *AKT1* E17K colorectal carcinoma cohort as *BRAF*-mutant microsatellite-stable colorectal carcinoma has been associated with more aggressive disease and poor survival (16).

Unlike *AKT1* E17K– and *PIK3CA* H1047R/L/Y–mutated colorectal carcinoma, *PIK3CA* R88Q–, E542K–, and E545K/G/D–mutated colorectal carcinoma had a very low incidence of *BRAF* V600E co-mutation and relatively higher incidences of *KRAS* co-mutations. Recent work has shown that *PIK3CA* helical domain mutations in codons 542 and 545 require activation of the RAS–GTP pathway, while kinase domain mutations in codon 1047 do not (17). This may explain why we found that the *PIK3CA* exon 10–mutant cases had more *RAS* and fewer *BRAF* co-mutations in comparison with the *PIK3CA* exon 21–mutant cases.

These associations are in line with previous work by Day and colleagues (4) for *PIK3CA* E542, E545, and H1047 mutations. To our knowledge, this is also the first clinicopathologic report of *PIK3CA* R88Q in colorectal carcinoma. *PIK3CA* R88Q mutations have previously been reported in endometrial carcinoma. This mutation is thought to activate PI3K through the formation of a hydrogen bond with D746 that results in conformational change of the encoded protein and increased kinase activity (18). While our numbers were too low for statistical analysis, the histologic and molecular profile of this subset seems more similar to helical domain (E542 and E545) mutant cases than kinase domain (H1047) mutant cases.

Our data also show that PTEN loss of expression and activating mutations within other areas of the *PI3K* mutation are not mutually exclusive as approximately one third of all *AKT1* or *PIK3CA* mutants lost PTEN expression. We found no difference in downstream activation of p-PRAS40 by IHC between *AKT1* E17K, *PIK3CA* helical domain, *PIK3CA* kinase domain, *RAS*, and *BRAF* mutants in this study.

A potential bias of our study is the preponderance of metastatic cases. In the vast majority of colorectal carcinoma submitted for clinical molecular analysis at our institution, testing is performed to determine candidacy for anti-EGFR therapy, and, therefore, almost all of our cases (including all 17 *AKT1* E17K) were late stage. Whether these results are applicable across all stages remains to be seen. Another shortfall pertains to the material tested: The technical sensitivity of our assay is 10%, thus false negatives due to low percentages of tumor cells or subclonal mutations may not be excluded. Finally, although the correlation between MSI testing by PCR and mismatch repair protein expression is very good, rare cases with mismatch repair abnormalities may be missed by either method.

In summary, the mutation *AKT1* E17K in colorectal carcinoma is associated with mucinous morphology, pulmonary metastases, and co-mutation of *BRAF* V600E. They occur in mutual exclusivity of *PIK3CA* mutations. Although *AKT1* E17K mutations, even without concurrent BRAF or RAS mutations, seem to confer primary resistance to anti-EGFR therapy in our limited series, this observation warrants further studies. In addition, it may serve as a targetable alteration in both RAS/RAF–mutated and RAS–RAF wild-type colorectal carcinoma.

Disclosure of Potential Conflicts of Interest

J.F. Hechtman is a consultant/advisory board member for Navigant. R. Yaeger is a consultant/advisory board member for Amgen. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: J.F. Hechtman, M. Ladanyi, M.E. Arcila
Development of methodology: J.F. Hechtman, J.T. Huse, J. Shia, M.E. Arcila
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.F. Hechtman, J.T. Huse, L. Borsu, R.D. Yaeger, J. Shia, E. Vakiani, M.E. Arcila
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.F. Hechtman, J. Sadowska, J.T. Huse, R.D. Yaeger, J. Shia, E. Vakiani, M.E. Arcila
Writing, review, and/or revision of the manuscript: J.F. Hechtman, J. Sadowska, J.T. Huse, R.D. Yaeger, J. Shia, E. Vakiani, M. Ladanyi, M.E. Arcila
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Sadowska, M.E. Arcila
Study supervision: M. Ladanyi, M.E. Arcila

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