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Retrospective Cohort Study

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Somatic mutations in FAT cadherin family members constitute an underrecognized subtype of colorectal adenocarcinoma with unique clinicopathologic features

Wang LL *et al.* Mutation of FAT family genes in colorectal adenocarcinoma

Abstract

BACKGROUND

The FAT cadherin family members (FAT1, FAT2, FAT3 and FAT4) are conserved tumor suppressors that are recurrently mutated in several types of human cancers, including colorectal carcinoma (CRC).

AIM

To characterize the clinicopathologic features of CRC patients with somatic mutations in FAT cadherin family members.

METHODS

We analyzed 526 CRC cases from The Cancer Genome Atlas PanCancer Atlas dataset. CRC samples were subclassified into 2 groups based on the presence or absence of somatic mutations in *FAT1*, *FAT2*, *FAT3* and *FAT4*. Individual clinicopathological data were collected after digital slide review. Statistical analysis was performed using *t* tests and chi-square tests.

RESULTS

This CRC study cohort had frequent mutations in the *FAT1* (10.5%), *FAT2* (11.2%), *FAT3* (15.4%) and *FAT4* (23.4%) genes. Two hundred CRC patients (38.0%) harbored somatic mutations in one or more of the FAT family genes and were grouped into the FAT mutated CRC subtype. The FAT-mutated CRC subtype was more commonly located on the right side of the colon (51.0%) than in the rest of the cohort (30.1%, $P < 0.001$). It showed favorable clinicopathologic features, including a lower rate of positive lymph nodes (pN1-2: 33.5% *vs* 46.4%, $P = 0.005$), a lower rate of metastasis to another site or organ (pM1: 7.5% *vs* 16.3%, $P = 0.006$), and a trend toward an early tumor stage (pT1-2: 25.0% *vs* 18.7%, $P = 0.093$). FAT somatic mutations were significantly enriched in microsatellite instability CRC (28.0% *vs* 2.1%, $P < 0.001$). However, FAT somatic mutations in microsatellite stable CRC demonstrated similar clinicopathologic

behaviors, as well as a trend of a better disease-free survival rate (hazard ratio = 0.539; 95% confidence interval: 0.301-0.967; log-rank $P = 0.073$).

CONCLUSION

FAT cadherin family genes are frequently mutated in CRC, and their mutation profile defines a subtype of CRC with favorable clinicopathologic characteristics.

Key Words: FAT cadherin family genes; Colorectal adenocarcinoma; Clinicopathologic features; Prognosis; The Cancer Genome Atlas

Wang LL, Zheng W, Liu XL, Yin F. Somatic mutations in FAT cadherin family members constitute an underrecognized subtype of colorectal adenocarcinoma with unique clinicopathologic features. *World J Clin Oncol* 2022; In press

Core Tip: Colorectal carcinoma (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide. In this study, we aimed to characterize the clinicopathologic features of CRC patients with somatic mutations in FAT cadherin family members. CRC cases have frequent mutations in FAT family genes. The FAT-mutated CRC subtype is more commonly located on the right side of the colon and shows favorable clinicopathologic features, including a lower rate of positive lymph nodes and a lower rate of metastasis to another site or organ, suggesting that the *FAT* somatic mutation is a potentially independent prognostic factor in CRC.

INTRODUCTION

Colorectal carcinoma (CRC) is the third most common cancer and the second leading cause of cancer-related deaths worldwide, with more than 1.9 million new cases and 935000 deaths in 2020^[1]. Except for a few CRC cases (5%-10%) with inherited gene mutations, most CRC cases occur sporadically and exhibit chromosomal instability that leads to changes in chromosome numbers and structure, featuring aneuploidy, loss of

heterozygosity, subkaryotypic amplification, and chromosomal rearrangement. Along with karyotypic abnormalities, mutations in specific tumor suppressor genes and oncogenes, such as the adenomatous polyposis (*APC*) gene, tumor protein p53 (*TP53*) and *KRAS* proto-oncogene GTPase, also contribute to CRC tumorigenesis. Notably, mutation of the *APC* gene, which leads to the activation of Wnt/ β -catenin signaling, is an essential and early event in the development of CRC^[2,3].

Despite the well-defined genetic and epigenetic alterations in CRC initiation and progression, recent studies have shown that the Hippo pathway may interact with Wnt/ β -catenin signaling and play a crucial role in controlling intestinal stem cell proliferation and CRC development^[4]. The Hippo pathway is an emerging tumor suppressor pathway. As a proposed upstream component of the Hippo pathway, the atypical cadherin FAT acts as a receptor to activate the Hippo pathway^[5], and its mutation appears to be a recurrent event in human cancers in association with dysregulation of the Hippo pathway^[6].

The human FAT cadherin gene family comprises the *FAT1*, *FAT2*, *FAT3* and *FAT4* genes^[7-10]. The encoded proteins FAT1-4 are human homologs of *Drosophila* FAT, of which *FAT1* and *FAT4* have been reported to be involved in the regulation of planar cell polarity^[11] and tumor suppression^[12,13]. *FAT1* also promotes actin-mediated cell migration^[14,15] and plays a role in epithelial mesenchymal transition^[16]. Somatic mutations of FAT family genes have been detected in different human cancers, including squamous cell carcinoma of the head and neck (*FAT1*, *FAT2* and *FAT4*)^[17-20], breast cancer (*FAT1*)^[21], melanomas (*FAT4*)^[22], leukemia (*FAT1*)^[23,24], hepatocellular cancer (*FAT1*, *FAT4*)^[25,26], esophageal squamous cell carcinoma (*FAT1*)^[27-29], pancreatic cancer (*FAT1*, *FAT3* and *FAT4*)^[30,31], and gastric cancer (*FAT4*)^[32,33]. Alterations in FAT family genes are associated with tumorigenesis and prognosis. For instance, upregulation of the *FAT1* gene is associated with poor prognosis and early relapse in acute lymphoblastic leukemia patients^[24] and invasive progression of ductal carcinoma in situ^[21], while loss of *FAT4* is associated with invasiveness in gastric cancer^[34]. Until now, the role of FAT family genes in CRC tumorigenesis has not been well studied. In

this study, we characterized the clinicopathologic features of FAT family gene mutations in CRC patients.

MATERIALS AND METHODS

Study design

In total, 526 CRC cases were selected from The Cancer Genome Atlas (TCGA) PanCancer Atlas dataset. cBioPortal (<https://www.cbioportal.org/>) was used to download whole-exome somatic mutation data and clinical information. There are certain sample inclusion criteria for the TCGA PanCancer Atlas on colorectal adenocarcinoma. The biospecimens were collected from newly diagnosed colorectal adenocarcinoma patients undergoing surgical resection, regardless of histologic grade or tumor stage. The patients had not received prior chemoradiation therapy. The histological sections contained an average of 60% tumor cells with less than 20% necrosis^[35].

In the TCGA PanCancer Atlas dataset, the somatic mutation profiles of *FAT1*, *FAT2*, *FAT3* and *FAT4* were analyzed for each tumor. Furthermore, the CRC cases were categorized into two groups based on their mutational status on FAT family genes: The cases with mutant *FAT1-4* and the cases with wild-type *FAT1-4*. Standard demographic and clinicopathological data were retrieved for each patient, including age, sex, tumor location, pT stage, pN stage, pM stage, differentiation grade, tumor type, lymphovascular invasion, month of disease-free survival (DFS) and overall survival (OS).

Statistical analyses

Demographic and clinicopathological details were stratified according to *Fat1-4* mutation. Quantitative and qualitative variables were expressed as the means \pm SD and the frequencies. Comparisons between the groups were analyzed with *t* tests and chi-square tests. DFS and OS were analyzed using the Kaplan-Meier method, and the log-rank test was used to assess differences. The figure was prepared using GraphPad

Prism 9 software (GraphPad Software, San Diego, California, United States). *P* values less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

The study included 526 patients with CRC from TCGA PanCancer Atlas Dataset. The mean age of the patients was 65.8 years (SD 13.0 years; range: 31-90 years). Based on the available clinicodemographic information, two hundred fifty-two patients were female, and two hundred seventy-two patients were male. Of them, 254 (48.3%) patients had left-sided colon cancer, and 197 (37.5%) patients had right-sided colon cancer. The majority (72.4%) of the CRCs were moderately differentiated adenocarcinomas. The detailed demographics, histopathologic stage and features are summarized in Table 1.

Somatic mutations of FAT family genes in CRC

Among the 526 CRC cases, 200 (38.0%) patients harbored one or more somatic mutations of the FAT cadherin family genes, including mutations in the *FAT1* (10.5%), *FAT2* (11.2%), *FAT3* (15.4%), and *FAT4* (23.4%) genes. The somatic mutation types of the FAT family genes include missense mutation, nonsense mutation, splicing mutation, frameshift deletion, frameshift insertion and in-frame deletion, with missense mutation being the most common somatic mutation type (Table 2). Interestingly, these somatic mutations were significantly enriched in the extracellular cadherin domain (*FAT1*, 49.0%; *FAT2*, 63.4%; *FAT3*, 40.1%; *FAT4*, 57.8%) (Table 2).

Based on the presence or absence of somatic mutations in *FAT1-4* genes, these cases were subclassified into 2 groups in our study. The clinicopathologic features of these 2 subtypes are summarized in Table 3. In the FAT-mutated CRC subtype, the median patient age was 66.5 years (range: 33-90 years), and 102 (51.0%) patients were male. Compared with the rest of the cohort, the FAT-mutated CRC subtype was more commonly located on the right side of the colon (51.0% vs 30.1%, $P < 0.001$) and more commonly associated with favorable histopathologic features, including lower

pathological nodal stage (pN0: 66.5% vs 52.8%, $P = 0.005$), lower rate of metastasis to another site or organ (pM1: 7.5% vs 16.3%, $P = 0.006$), and a trend of lower pathological tumor stage (pT1-2: 25.0% vs 18.7%, $P = 0.093$).

FAT somatic mutations are enriched in microsatellite-*instable* CRC

Human FAT family genes encode large atypical cadherin proteins with a large number of cadherin repeats. Given the overlapping features found in the FAT-mutated CRC subtype and microsatellite-*instable* (MSI) CRC (right sided with favorable clinicopathological features), we further explored the association between FAT mutations and MSI. Interestingly, FAT somatic mutations were significantly enriched in MSI CRC (28.0% vs 2.1%, $P < 0.001$) (Table 3).

To control for confounding in the analysis, we focused on cases of microsatellite-*stable* (MSS) CRC. As shown in Table 1, the MSS CRC cases showed similar clinicodemographic and histologic features as the entire cohort. We also categorized the MSS CRC cases into 2 groups based on the mutation status of FAT family genes. Similar to the entire cohort we described earlier, the FAT-mutated MSS CRC subtype was also more commonly located on the right side of the colon (39.6% vs 28.8%, $P = 0.038$) and more commonly associated with favorable histopathologic features, such as a lower rate of metastasis to another site or organ (pM1: 9.0% vs 16.6%, $P = 0.038$). It also showed a trend of lower pathological tumor stage (pT1-2: 26.4% vs 19.1%, $P = 0.083$) and lower pathological nodal stage (pN0: 60.4% vs 52.7%, $P = 0.079$) (Table 3). Therefore, even though it is enriched in MSI CRC, the FAT somatic mutation is a potentially independent prognostic factor in CRC.

The median DFS for CRC patients was 26.0 mo (0.5-148.0 mo), and the OS was 21.0 mo (0-148.0 mo). Consistent with the favorable pathologic features, the FAT-mutated MSS CRC subgroup showed a trend toward a better DFS rate [hazard ratio (HR) = 0.539; 95% confidence interval (CI): 0.301-0.967; log-rank $P = 0.073$]. However, FAT mutation status did not show a significant impact on the OS rate (HR = 1.198; 95%CI: 0.770-1.864; log-rank $P = 0.440$) (Figure 1).

DISCUSSION

5 To our knowledge, this is the first study to assess the impact of somatic mutations in FAT family genes on clinicopathologic features, with an emphasis on prognosis in CRC patients. Our study shows that somatic mutations in FAT family genes are associated with favorable clinicopathologic features, including a lower rate of lymph node and distal metastasis. It also showed a trend toward a lower tumor stage with a relatively favorable DFS.

In addition to the *APC- β -catenin* pathway, which represents the most prominent signaling pathway in CRC, components of the Hippo pathway have been reported to be involved in CRC tumorigenesis^[36-40] and have been proposed as prognostic factors in CRC^[41-44]. As an upstream organizer and activator of the Hippo pathway^[6], FAT family genes have emerged as an important mechanism that orchestrates epithelial development as well as human cancer initiation and progression. The FAT family genes (*FAT1-4*) encode atypical cadherins that contain multiple extracellular cadherin repeats, laminin G motifs and EGF-like motifs^[45]. Among these, *FAT1* and *FAT4* are relatively well studied. Loss of *FAT4* expression has been reported in some primary breast cancers and breast cancer cell lines^[46]. Low *FAT4* expression was also observed in gastric cancers and was associated with a poor prognosis, including high pathologic T stage, an increase in perineural invasion, high lymph node metastasis and reduced DFS^[47]. Similarly, a study reported recurrent *FAT1* mutations in multiple human cancers, including glioblastoma, CRC, and head and neck cancer, and *FAT1* mutations affected patient survival by promoting Wnt signaling and tumorigenesis^[48]. Our study demonstrates that somatic mutations in FAT family genes are frequent recurrent events in CRC and that FAT mutations are associated with favorable clinicopathologic features. These somatic mutations are highly enriched in the extracellular cadherin domains (Table 2). FAT proteins are large single transmembrane receptors characterized by 32-34 extracellular cadherin repeats. These cadherin repeats contain highly conserved binding sites for proteins, such as β -catenin and p120-catenin,

which are important for the FAT protein to execute its role in migration, polarity and cell adhesion by linking it to the actin cytoskeleton.

Our study also revealed the significant enrichment of FAT-mutated CRC (28.0%) in the MSI subgroup. However, the clinicopathologic characteristics in FAT-mutated MSS CRC are quite compatible with the entire FAT-mutated CRC cohort in our study, suggesting that MSI only partially contributes to its pathologic features and clinical outcomes. Interestingly, FAT-mutated MSS CRC cases showed a trend of favorable DFS but not OS. The underlying mechanisms of this discrepancy are currently unclear. Notably, DFS does not always correlate with OS in CRC, such as in the case of liver-only metastatic CRC^[49].

Similar to the findings in our study, Wang *et al*^[33] reported a superior prognosis in gastric adenocarcinoma with FAT family gene mutations. In their study, FAT gene mutations were significantly associated with better progression-free survival and OS, which was likely attributed to the significantly higher tumor mutational burden and an inflamed tumor microenvironment^[33]. Whether the tumor microenvironment plays a similar role in CRC still awaits further investigation.

Our study has several limitations. First, our findings were obtained from a bioinformatics study on somatic mutation profiles through the TCGA PanCancer Atlas dataset. The protein expression levels of individual FAT family members were not systemically examined in the study, and the underlying molecular mechanisms related to the prognostic role of the FAT family in colorectal cancer need further experimental validation. Second, all the patients in the study were untreated, with no therapy response data and a short follow-up. Therefore, the evaluation of advanced-stage CRC is relatively limited. Third, we tried to address the impact of MSI status, a confounding factor, by analyzing the MSS samples. However, there are still additional potential confounding factors, such as histopathological subtypes, *TP53* mutation status, and intratumoral spatial and temporal heterogeneity. The ability of our study to address these potential confounding factors is hampered by intrinsic limitations of the TCGA database, and the landmark cancer program heavily focused on cancer genomics

datasets. A randomized, large-scale clinical cohort is necessary to validate our conclusion and to establish somatic mutations in FAT family genes as independent prognostic factors for CRC in future studies.

CONCLUSION

In summary, our study shows that somatic mutations in FAT family genes are recurrent genetic events detected in approximately 38% of CRC cases and therefore represent an underrecognized subtype of CRC. The FAT-mutated CRC subtype shows unique clinicopathologic features, including a right-side location, a lower rate of positive lymph nodes, a lower rate of metastasis to another site or organ, and a trend toward favorable DFS. Our study suggests that somatic mutations in FAT family genes are potential prognostic biomarkers for CRC.

ARTICLE HIGHLIGHTS

Research background

² The human FAT cadherin gene family comprises the FAT1, FAT2, FAT3 and FAT4 genes. Somatic mutations of FAT family genes have been detected in different human cancers.

Research motivation

Until now, the role of FAT family genes in colorectal carcinoma (CRC) tumorigenesis has not been well studied. In this study, we characterized the clinicopathologic features of FAT family gene mutations in CRC patients.

Research objectives

¹ In total, 526 CRC cases were selected from The Cancer Genome Atlas PanCancer Atlas dataset.

Research methods

CRC cases were categorized into two groups based on their mutational status on FAT family genes: The cases with mutant *FAT1-4* and the cases with wild-type *FAT1-4*. Standard demographic and clinicopathological data were retrieved for each patient, including age, sex, tumor location, pT stage, pN stage, pM stage, differentiation grade, tumor type, lymphovascular invasion, month of disease-free survival and overall survival.

Research results

¹ The FAT-mutated CRC subtype is more commonly located on the right side of the colon and shows favorable clinicopathologic features, including a lower rate of positive lymph nodes and a lower rate of metastasis to another site or organ.

Research conclusions

¹ FAT cadherin family genes are frequently mutated in CRC, and their mutation profile defines a subtype of CRC with favorable clinicopathologic characteristics.

Research perspectives

FAT somatic mutation is a potentially independent prognostic factor in CRC.

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