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Clinical significance of *BRAF* non-V600E mutations in colorectal cancer: a retrospective study of two institutions

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ABSTRACT

Background: Recent advances in next-generation sequencing have enabled the detection of *BRAF* V600E mutations as well as *BRAF* non-V600E mutations in a single assay. The present work aimed to describe the clinicopathological characteristics and clinical outcome of the *BRAF* non-V600E mutant-type in colorectal cancer (CRC).

Patients and methods: CRC samples from 111 Stage IV patients were analyzed for somatic mutations using a 415-gene comprehensive genomic sequencing panel. Patients were classified according to *BRAF* status as wild-type, V600E mutant-type, or non-V600E mutant-type. Differences between clinicopathological characteristics and genetic alterations were analyzed among the three groups. Overall survival (OS) and the response to anti-EGFR therapy were also analyzed.

Results: Comprehensive genomic sequencing revealed that 98 patients (88%), 7 patients (6%), and 6 patients (6%) were wild-type, V600E mutant-type, and non-V600E mutant-type, respectively. Non-V600E mutant-type tumors were frequently left-sided (83%), while V600E mutant-type tumors were frequently right-sided (86%; $P = 0.025$). Non-V600E mutant-type showed better OS than V600E mutant-type ($P = 0.038$), with no significant difference compared with wild-type tumors. The two patients with non-V600E mutations who

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underwent repeated metastasectomies showed no evidence of disease at final follow-up. Regarding the efficacy of anti-EGFR therapy, the patient with an I326V mutation had progressive disease (+115%) despite no genetic alterations detected in the EGFR pathway that could drive resistance, suggesting an alternate resistance mechanism.

Conclusions: Non-V600E mutant-type is more likely to be left-sided and demonstrates better OS than V600E mutant-type. Further preclinical and clinical investigations are needed to clarify the role of non-V600E mutations in CRC.

Introduction

The *BRAF* gene encodes BRAF, a member of the RAF family of serine/threonine-protein kinases, which binds directly to RAS following stimulation by tyrosine kinases (TKs) such as the EGFR receptor.[1] BRAF is activated by dimerizing with BRAF or CRAF and plays a role in regulating the MEK/ERK signaling pathway, which affects cell proliferation and cell survival.[2] Mutations in *BRAF* can result in activation of the MEK/ERK signaling pathway and are associated with various cancers.[3] *BRAF* V600E mutations are one of the most frequently identified cancer-causing mutations in melanoma, as well as in various other cancers, including thyroid carcinoma, biliary cancer, and colorectal cancer (CRC).[4,5]

Approximately 5 to 9% of CRCs have a *BRAF* V600E mutation and are recognized as a distinct molecular subtype of CRC.[6,7] A retrospective analysis of patients with metastatic CRC found two-thirds of patients with a *BRAF* V600E mutation had right-sided colon cancer, with an increased incidence of peritoneal and distant lymph node metastases but fewer pulmonary metastases.[8] Multiple studies have shown that the *BRAF* V600E mutation is associated with poor response to anti-EGFR therapy in later lines of therapy[9,10] as well as poor prognosis in metastatic CRC.[11-17] Hence, *BRAF* genotyping is recommended in metastatic CRC, especially before initiation of anti-EGFR therapy.[6,7]

Direct sequencing has long been the preferred technique to detect *BRAF* mutations and is still widely used. However, in the past 10 years, next-generation sequencing (NGS) projects, such as The Cancer Genome Atlas, have profiled genomic changes in many cancers, including CRC.[18] Comprehensive genomic sequencing (CGS) using NGS enables the detection of numerous genetic alterations and is expected to drive a paradigm shift from pathologic microscopic-based approaches to genetic signature-based diagnoses.[19]

CGS allows the simultaneous detection of *BRAF* V600E mutations as well as *BRAF* non-V600E mutations in a single assay. Approximately 200 *BRAF* mutant alleles have been identified in human cancer. Many mutations result in activation of MEK/ERK signaling but through different mechanisms, which will dictate the sensitivity to therapeutic inhibitors of the pathway.[20,21] Thus, it is clear that expanded mutational information can and should influence clinical practice; however, for the majority of the mutations, the clinical, therapeutic, and prognostic implications remain uncertain.

Although preclinical and clinical data on *BRAF* non-V600E mutations in CRC were gradually accumulated,[22-24] their clinical relevance was yet to be clarified. The present work aimed to describe clinicopathological characteristics and clinical outcome of the *BRAF* non-V600E mutant-type compared with *BRAF* wild-type and *BRAF* V600E mutant-type.

Materials and methods

Patients

This retrospective study was performed in accordance with the Helsinki Declaration, and the Ethics Committee of the Niigata University School of Medicine approved the study protocol. A total of 111 patients diagnosed with stage IV CRC (AJCC, seventh edition)[25] who underwent a primary tumor resection between 2009 and 2015 at the Niigata University Medical and Dental Hospital or Niigata Cancer Center Hospital were randomly selected and enrolled in this study. Patients with familial adenomatous polyposis or inflammatory bowel disease were excluded. Of the 111 patients, metastasis to the liver, lung, peritoneum, and other sites at initial assessment were identified in 88, 34, 22, and 23 patients, respectively. Thirty-seven and 74 patients were diagnosed with resectable and unresectable metastatic disease, respectively, at initial assessment. Patients were classified according to residual tumor status. Patients who achieved R0 resection of both the primary lesion and distant metastasis were classified as "R0", while patients for whom R0 resection could not be achieved were classified as "R2". Typically, chemotherapy was administered according to the Japanese Society for Cancer of the Colon and Rectum guidelines.[26]

CGS analysis of primary tumors

Archival tissue in the form of formalin-fixed, paraffin-embedded tumor or unstained tissue sections obtained during primary tumor resection were used for CGS. Tumor content was evaluated by an independent pathologist for each sample using hematoxylin and eosin-stained slides to ensure >50% tumor content. Where applicable, unstained slides were macrodissected to enrich for tumor content, and DNA was extracted using a BioStic formalin-fixed, paraffin-embedded Tissue DNA Isolation Kit (Mo Bio Laboratories, Inc, Carlsbad, CA).[27] All sample preparations, CGS, and analytics were performed in a CLIA/CAP-accredited laboratory (KEW Inc, Cambridge, MA). DNA fragment (50-150 ng) libraries were prepared and enriched for the CancerPlex 415-gene panel (KEW Inc),[28-30] a large and clinically validated panel of 415 genes enriched for coding regions and selected introns of known cancer-related genes. Sequencing was