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Braf gene mutations characteristics on colorectal cancer patients by Diatech pharmacogenetics instrument: Results at hue central hospital and literature review

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Abstract
BRAF mutations are rare, but these mutations can cause many serious health problems. BRAF mutations occur in colorectal cancer, thyroid cancer, melanoma, ovarian tumor, etc. Colorectal cancer with BRAF gene mutation at the V600E has a worse prognosis than those without mutation. Hue Central Hospital, using a Real-time PCR instrument of Diatech Pharmacogenetics Company, detected 2 of 62 cases of BRAF gene mutation at V600E with lymph node metastasis as soon as cancer was detected. The purpose of the article is to record data and review the literature to study more on this type of clinical disease.

Keywords: Diatech Pharmacogenetics, characteristics, colorectal cancer, cancer patients

Introduction

Overview

Colorectal cancer is the main cause of cancer death in Vietnam as well as abroad. Recent studies have revealed the mechanism by which genes changes (Mutations) lead to progression from normal colon tissues to true carcinoma.

According to Alberto Fernández-Medarde *et al.*, the importance of RAS in regulating cell proliferation is illustrated by the presence of K-RAS-activating mutations in approximately one-third of human cancers., including up to 50% of colorectal cancers [1].

Furthermore, 20% of colorectal tumors with wild-type KRAS carry BRAF-activating mutations. The addition of mutations between KRAS and BRAF play a central role for the RAS-RAF-MAPK pathway in the pathogenesis of colorectal cancer.

Genes of the RAF family encode kinases that are regulated by Ras and mediate cellular responses to growth signals. Activating mutations in a BRAF gene, have been found in a high percentage of melanomas and in a small fraction of other cancers.

The RAS family includes three isoforms (H-RAS, K-RAS and N-RAS) with high degree of sequence identity. The RAS signaling cascade constitutes a major pathway controlling cell proliferation, and RAS proteins can integrate extracellular signals from different types of receptors.

The RAS/RAF/MEK/ERK signaling chain, also known as the MAPK (mitogen-activated protein kinase) pathway, is involved in cell proliferation, differentiation, survival, and apoptosis. It receives input from multiple sources including internal metabolism and altered DNA damage pathways and protein levels, as well as through signals from external growth factors, cell-matrix interactions, and communication from other cells [2].

Multiple signals activate RAS (KRAS, NRAS and HRAS), a class of GTPase. This in turn, activates downstream RAF protein kinases (ARAF, BRAF and CRAF). The predominant substrates of the RAF kinase are the MAPK/ERK, MEK1 and MEK2 kinases. ERKs phosphorylate a variety of substrates, including many transcription factors that regulate several key cellular activities.

The RAF protein is made up of three conserved regions: CR1, CR2, and CR3. CR1 and CR2 are located at the N-terminus. CR1 serves as the primary binding domain for RAS; CR2 is the regulatory domain. CR3 is located at the C-terminus and functions as the catalytic kinase domain.

CR3 contains two regions important for RAF activation: The activating and regulatory regions [5]. Of the RAF family of protein kinases, BRAF is the most frequently mutated and remains the most potent activator of MEK. The BRAF protooncogene, which encodes for the protein kinase BRAF, is located on chromosome 7 (q34) and consists of 18 exons. There have been more than thirty BRAF mutations identified to date, occurring at various frequencies. The most common is the BRAF V600E (MT) mutant, which corresponds to the conversion of thymine to adenine at position 1799, resulting in a substitution of valine by glutamate at position 600 of the protein [5]. The V600E mutation accounts for more than 85% of BRAF mutations in melanoma, more than 50% of mutations in non-small cell lung cancer, and more than 95% of mutations in cholangiocarcinoma and hairy cell leukemia. It accounts for more than 90% of BRAF mutations in colorectal cancer (CRC). Other BRAF mutations include R461I, I462S, G463E, G463V, G465A, G465E, G465V, G468A, G468E, N580S, E585K, D593V, F594L, G595R, L596V, T598I, V599D, V599E.

BRAF mutations have been found in 7–10% of patients with metastatic CRC. The BRAF MT CRC has been associated with a specific phenotype in many studies and meta-analyses and is specifically associated with the BRAF V600E mutation. BRAF tumors are more common in women and in patients > 70 years of age. BRAF is not related to age of diagnosis under 60 years.

BRAF mutations are more common in proximal colon tumors and are rarely found in the left colon. Histopathology is also variable, with 60% of BRAF MT tumors poorly differentiated and a higher incidence of mucinous pathology. There is an association with larger primary tumors. The BRAF MT CRC is also associated with a high rate of peritoneal metastasis and less disease confined to the lungs and liver. In contrast, most non-V600 mutations were more likely to be lower and left-sided tumors with higher survival, with the exception of similarly active codon 601/597 mutations. such as V600E MT CRC.

BRAF represents a therapeutic target in cancer, unlike KRAS, which is a relatively unidirectional MEK-ERK agent. Inhibition of BRAF with vemurafenib (PLX4032) has been shown to significantly benefit patients with unresectable or metastatic BRAF V600E MT melanoma, improving survival and OS progression, with a response rate of 48%.

In contrast, the inhibition of BRAF in mCRC was disappointing. An extended phase II study examined vemurafenib in patients with BRAF MT mCRC who had at least one prior line of therapy. Of the 21 treated patients, 1 had a partial response and another 7 had stable disease according to the RESIST criteria. The average PFS is only 2.1 months, and the ORR is 5%. Despite indications of efficacy, the authors concluded that single-agent vemurafenib did not show any clinically meaningful activity in patients with BRAF V600E MT mCRC.

We carry out this study with the objective to study the pathologic features of colorectal cancer with BRAF mutations at the V600E and to review the literature on the pathogenesis and prognosis of the disease.

Report of clinical cases

From Jan 1st, 2022, to Feb 10th, 2023, we have done the test at Hue Central Hospital. The NRAS, KRAS, and BRAF genomic mutations on 62 colorectal cancer cases are tested and 2 cases which are positive with mutation in BRAF – V600E had been detected. Especially, when these two patients were found to have colon cancer, they had lymph nodes metastases.

Table 1: Rate of BRAF gene mutation in colorectal cancer at hue central hospital

	Number of cases	Percent (%)
BRAF mutation at V600E	2	3,2
Total	62	100

Table 2: Some pathological characteristics in two patients with BRAF mutations

	Case 1	Case 2
Age	35	51
Sex	Female	Male
Histology type	Adenocarcinoma, moderately differentiated, invasive	Adenocarcinoma, moderately differentiated, invasive
Metastasis to lymph node	Yes	Yes
Gene mutation	BRAF – V600E	BRAF – V600E
Tumor site	Rectum	Rectum
Tumor size	4x4x6cm	3x4x5cm
Others	Non - smoking	Smoking, 10 cigarettes per day

Discussion

1. Regarding the characteristics of colorectal cancer with mutations in the BRAF gene: In two colorectal cancer patients with mutations in the BRAF gene, both tumors were detected at clinical stage 3 or higher, and nodal metastases were detected simultaneously. The ratio of colorectal cancer with BRAF gene mutation is 3.2%.

diatech pharmacogenetics

Platform: RT800-96_EasyPGX qPCR instrument 96

Kit: RT022_EasyPGX ready BRAF

Software version: 4.0.14

Experiment description:

Detailed Result Report

Sample	Assay	Result
NGUYỄN THỊ THANH T	BRAF V600E/Ec	MUT

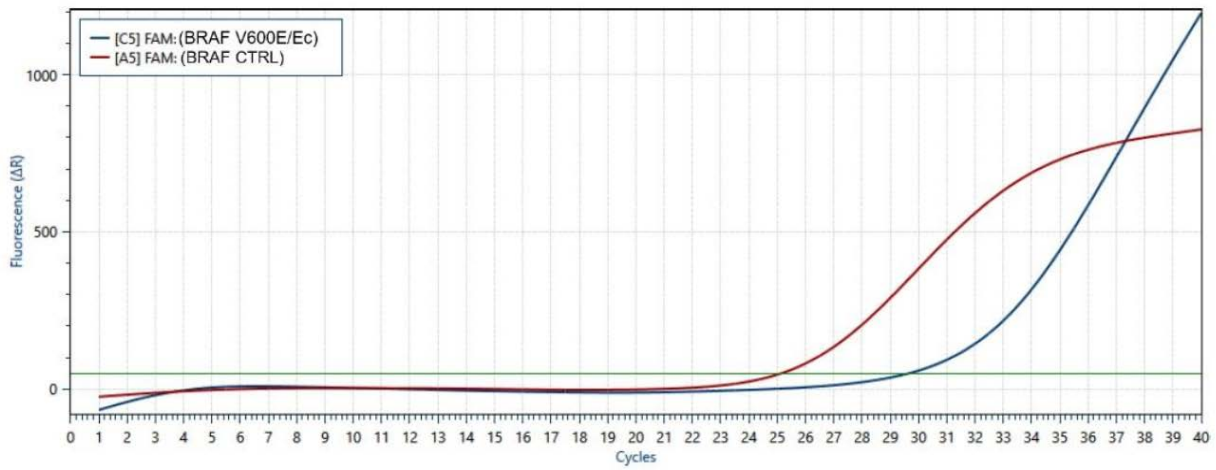


Fig 1: Data Visualization of BRAF - V600E/Ec mutation positive - Case 1

diatech pharmacogenetics

Platform: RT800-96_EasyPGX qPCR instrument 96

Kit: RT021_EasyPGX ready BRAF

Software version: 4.0.14

Experiment description:

Detailed Result Report

Sample	Assay	Result
ĐỖ VĂN M	BRAF V600E/Ec	MUT

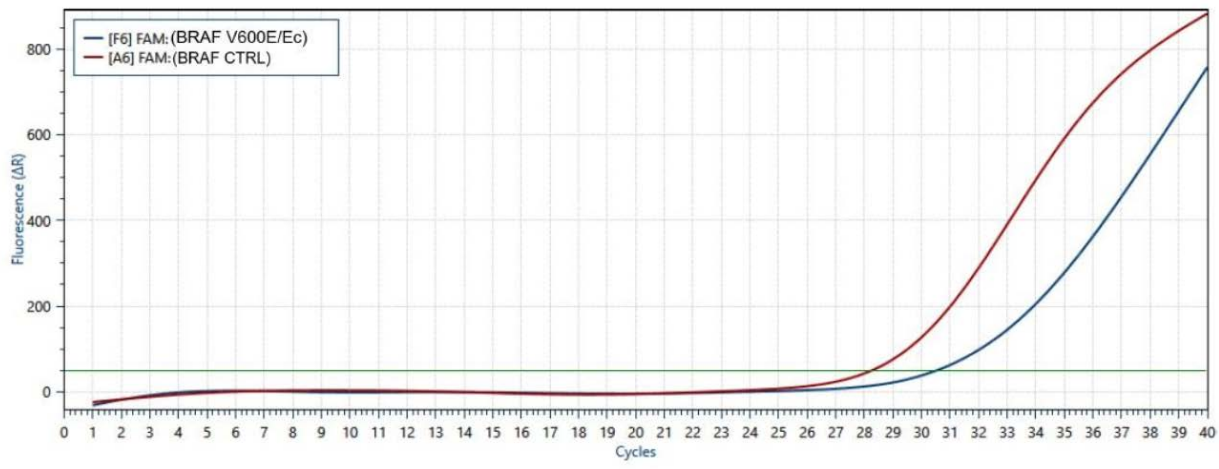
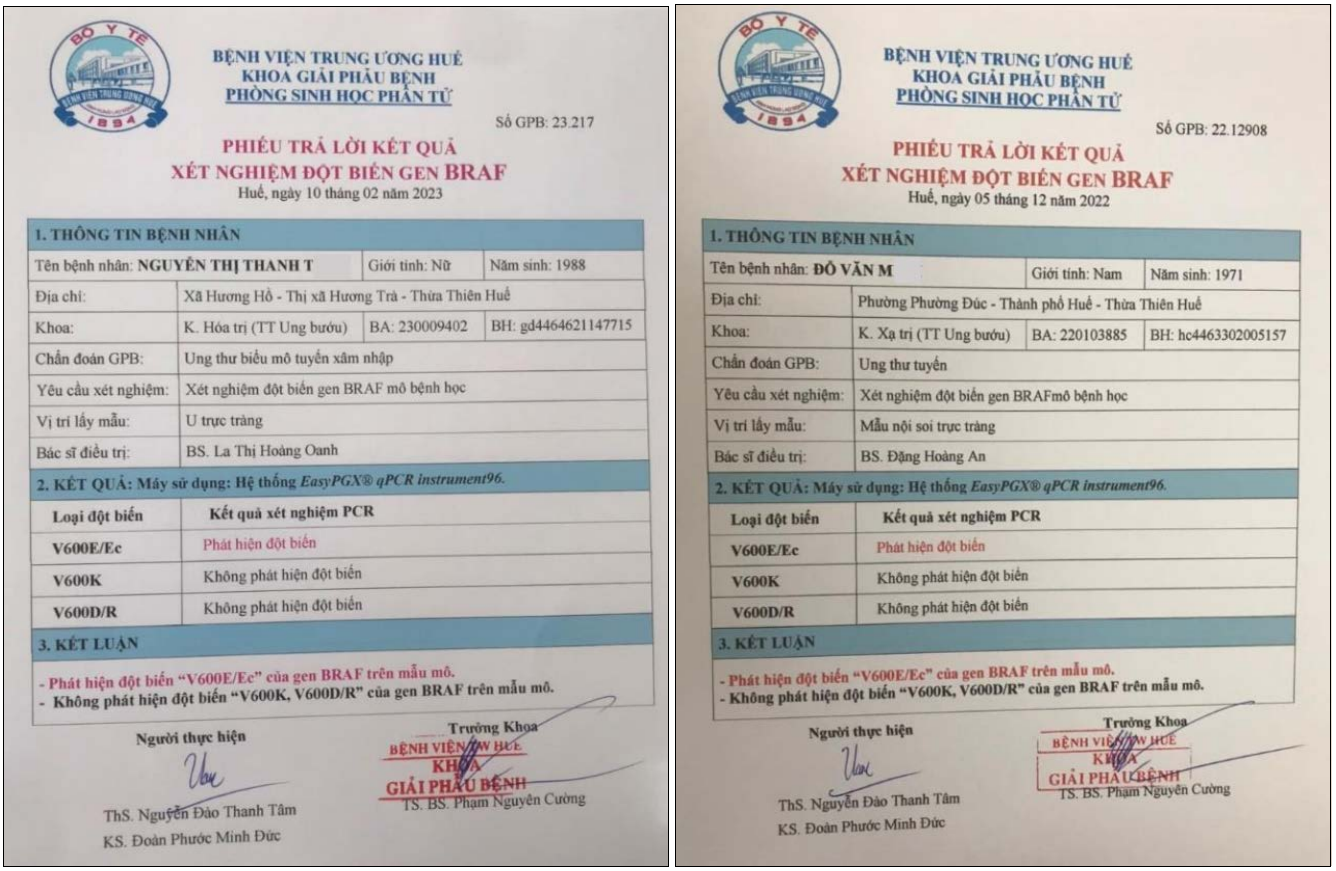


Fig 2: Data Visualization of BRAF - V600E/Ec mutation positive - Case 2

According to a study by Lee-Jen Luu *et al.*, colorectal cancer has mutations in the BRAF-V600E gene found in nearly 10% of patients with advanced colorectal cancer. Despite major improvements in survival for advanced colorectal cancer overall, patients with BRAF mutations have a very poor prognosis often with a median survival of less than 12 months.

It is important for clinicians to be aware of this subtype because treatment approaches are different. Treatment options other than standard chemotherapy are critical to achieving better outcomes, and the role of anti-EGFR therapy alone remains controversial. Current trials evaluating combinations of molecularly targeted agents have shown some promises.



BỆNH VIỆN TRUNG ƯƠNG HUẾ
KHOA GIẢI PHẪU BỆNH
PHÒNG SINH HỌC PHÂN TỬ

Số GPB: 23.217

PHIẾU TRẢ LỜI KẾT QUẢ
XÉT NGHIỆM ĐỘT BIẾN GEN BRAF
Huế, ngày 10 tháng 02 năm 2023

1. THÔNG TIN BỆNH NHÂN			
Tên bệnh nhân: NGUYỄN THỊ THANH T	Giới tính: Nữ	Năm sinh: 1988	
Địa chỉ:	Xã Hương Hồ - Thị xã Hương Trà - Thừa Thiên Huế		
Khoa:	K. Hóa trị (TT Ung bướu)	BA: 230009402	BH: gd4464621147715
Chẩn đoán GPB:	Ung thư biểu mô tuyến xâm nhập		
Yêu cầu xét nghiệm:	Xét nghiệm đột biến gen BRAF mô bệnh học		
Vị trí lấy mẫu:	U trực tràng		
Bác sĩ điều trị:	BS. La Thị Hoàng Oanh		

2. KẾT QUẢ: Máy sử dụng: Hệ thống *EasyPGX® qPCR instrument96.*	
Loại đột biến	Kết quả xét nghiệm PCR
V600E/Ec	Phát hiện đột biến
V600K	Không phát hiện đột biến
V600D/R	Không phát hiện đột biến

3. KẾT LUẬN

- Phát hiện đột biến "V600E/Ec" của gen BRAF trên mẫu mô.
- Không phát hiện đột biến "V600K, V600D/R" của gen BRAF trên mẫu mô.

Người thực hiện
ThS. Nguyễn Đào Thanh Tâm
KS. Đoàn Phước Minh Đức

Trưởng Khoa
TS. BS. Phạm Nguyên Cường

BỆNH VIỆN TRUNG ƯƠNG HUẾ
KHOA GIẢI PHẪU BỆNH
PHÒNG SINH HỌC PHÂN TỬ

Số GPB: 22.12908

PHIẾU TRẢ LỜI KẾT QUẢ
XÉT NGHIỆM ĐỘT BIẾN GEN BRAF
Huế, ngày 05 tháng 12 năm 2022

1. THÔNG TIN BỆNH NHÂN			
Tên bệnh nhân: ĐỖ VĂN M	Giới tính: Nam	Năm sinh: 1971	
Địa chỉ:	Phường Phường Đúc - Thành phố Huế - Thừa Thiên Huế		
Khoa:	K. Xạ trị (TT Ung bướu)	BA: 220103885	BH: hc4463302005157
Chẩn đoán GPB:	Ung thư tuyến		
Yêu cầu xét nghiệm:	Xét nghiệm đột biến gen BRAFmô bệnh học		
Vị trí lấy mẫu:	Mẫu nội soi trực tràng		
Bác sĩ điều trị:	BS. Đặng Hoàng An		

2. KẾT QUẢ: Máy sử dụng: Hệ thống *EasyPGX® qPCR instrument96.*	
Loại đột biến	Kết quả xét nghiệm PCR
V600E/Ec	Phát hiện đột biến
V600K	Không phát hiện đột biến
V600D/R	Không phát hiện đột biến

3. KẾT LUẬN

- Phát hiện đột biến "V600E/Ec" của gen BRAF trên mẫu mô.
- Không phát hiện đột biến "V600K, V600D/R" của gen BRAF trên mẫu mô.

Người thực hiện
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KS. Đoàn Phước Minh Đức

Trưởng Khoa
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Fig 3: Pathology gene mutation reports of the two patients at Hue Central Hospital

In terms of BRAF gene characterization, the serine/ threonine BRAF protein kinase is an important player in the mitogen-activated protein kinase (MAPK)-mediated epidermal growth factor receptor (EGFR) pathway, where it is stimulated activated by RAS small GTPase. The power of BRAF, and its extension to other RAF isoforms (ARAF and CRAF), not only activates the MAPK pathway profoundly affecting cell growth, proliferation, and differentiation, but also affects other important cellular processes, such as cell migration (via RHO small GTPases), apoptosis (via regulation of BCL-2) and survival (via the HIPPO pathway). Thus, it is not surprising that BRAF was found to be constitutively activated by mutation in 15% of all known human cancers. BRAF has been reported to be mutated at several sites; however, the majority of BRAF mutations are V600E (1799T > One nucleotide change), which characterizes up to 80% of all BRAF mutations. This mutation results in amino acid changes that induce structural kinase activity to. Most BRAF mutations result either in the acquisition of novel phosphomimetics residues or in the release of an autoinhibitory construct imposed by the N-terminus, which enhances kinase domain dimerization, a process important for kinase activity. BRAF inhibitors have been developed by different companies. The most used are vemurafenib (marketed as Zelboraf by Roche) and dabrafenib (marketed as Tafinlar by GSK), but others exist as LGX818 (encorafenib; Novartis), XL281 (Exelixis) and CEP-32496 (Ambit Biosciences Corporation).

Regarding the mechanism of colorectal cancer transformation, according to David Barras, colon polyps can be classified into adenomatous polyps (~10%) and hyperplastic polyps (~90%) [3]. Hyperplastic polyps do not progress to CRC. Some polyps are called serrated polyps (WHO classification: ICD-O 8213/0) because of their serrated morphology. These polyps have long been considered non-malignant, but this view has since been challenged. Serrated polyps are further classified into different types: Serrated hyperplasia, traditional serrated adenomas (TSA) or sessile serrated adenomas (SSAs). TSA and SSA are considered premalignant. Epithelial transformation to TSA and SSA polyps is thought to be caused by BRAF mutations, thus identifying this mutation as an early event in the progression of CRC. Activation of the WNT pathway in tandem with inactivation of p53 and p16 is present only in the late stages of CRC development. BRAF mutant tumors are often right-sided, relapsing more frequently in women, at grade higher degree and is associated with microsatellite instability (MSI) and senescence. MSI is a form of genetic instability resulting from a lack of a mismatched repair apparatus and resulting

in hypervariability. MSI is believed to be the best prognostic factor in CRC with instability conferring a better prognosis. Interestingly, the deleterious effects caused by BRAF mutations were more pronounced in microscopically stable patients than in unstable (MSI) patients, although not different in statistics. The interaction between the BRAF state and the MSI state is a hotly debated topic. Proximal right CRC is associated with a worse prognosis. BRAF mutations are found to be abundant in proximal right tumors. The reason for this association is not fully understood. For tissue analysis for a more detailed description of the molecular mechanism and early discoveries related to BRAF, the reader will be redirected to a recent in-depth review.

2. On the prognostic role of BRAF mutation

Although the predictive role of KRAS mutations for cetuximab (an antibody that blocks EGFR) is well established, the predictive role of mutated BRAF is a subject of intense controversy. Several studies have compared that the effects of anti-EGFR are beneficial in BRAF mutants. However, until recently, there were no formal studies on the impact of BRAF mutation acquisition on anti-EGFR response (that is, by comparison with wild-type BRAF patients). Recently, such a study was achieved by performing a meta-analysis that grouped eight groups including 351 BRAF mutant patients, including patients with rabies BRAF. This analysis showed a prevalence of 351 patients with BRAF mutations. The risk of patients treated with an EGFR-blocking antibody (cetuximab or panitumumab) was independent of BRAF mutation status for overall survival (interaction trial P-value: 0.43) but almost significant for progression-free survival (interaction trial P-value: 0.07). The authors concluded that mutant BRAF did not predict benefit induced by therapies. Brought against EGFR. Similarly, another meta-analysis reported by Pietrantonio *et al.*, revealed that EGFR-blocking antibodies did not increase the efficacy of standard chemotherapy in BRAF-mutant patients. However, this study did not evaluate the difference in survival between wild-type BRAF and BRAF-mutant patients.

In contrast, the prognostic role of BRAF mutations in CRC is well established and is generally associated with a significantly worse prognosis. For example, in a study by author David Barras involved more than 1200 stage II and III colorectal cancer patients with BRAF mutations, have shown that BRAF mutations significantly affect overall survival (hazard ratio: 1.78 [1.15-2)., 76]; with P = 0.01), on the other hand, recurrence-free survival was not changed (Hazard Ratio: 1.30 [0.87-1.95]; with p=0. 21).

According to David Barras, most patients with metastatic colorectal cancer carry the BRAF-V600E mutation and have a shorter progression-free survival (PFS) and overall survival (OS) compared to wild-type BRAFs foolish. Since 2005, researchers have early recorded OS shortening in stage II-IV colorectal cancer with BRAF-V600E mutation. The expected median OS for stage IV colorectal cancer (mOS) is generally approximately 29-30 months. Meanwhile, the standard multi-chemotherapy regimen resulted in only a modest mOS of 12-14 months for the group of patients with the BRAF-V600E mutation [3].

For newly diagnosed metastatic colorectal cancer patients with a BRAF-V600E mutation, what is the optimal treatment approach? Up to this point, we do not have a clear answer. CEB was examined in a phase II trial (ANCHOR CRC) in previously untreated metastatic colorectal cancer patients with the BRAFV600E mutation [4]. The authors expressed disappointment in the results of the 4.9-month PFS early report (95% CI 4.4-8.1 months), suggesting that CEB alone is not enough. A subgroup analysis (N=16) in the phase III trial TRIBE showed that the combination of 5FU, leucovorin, oxaliplatin and irinotecan + bevacizumab resulted in an improvement of mOS by 19.0 months in the BRAF-V600E mutant group compared with chemotherapy. standard value [5]. However, this finding from TRIBE was not confirmed in a recent meta-analysis [6].

Conclusion

The BRAF mutations in colorectal cancer occur very rarely, less than 10%, but these mutations can cause serious health problems. BRAF mutations occur in colorectal cancer, thyroid cancer, melanoma, ovarian tumor, etc. Colorectal cancer with a BRAF gene mutation at the V600E score has a worse prognosis than those without the mutations. Hue Central Hospital had 2 of 62 cases of BRAF gene mutation V600E with lymph node metastasis as soon as cancer is detected. Currently both these cases are being actively treated and closely monitored.

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