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Relationship between KRAS mutation and diffusion tensor imaging features in brain metastases due to colorectal cancer; preliminary study

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Abstract

Identifying Kirsten rat sarcoma (KRAS) mutation status is important for metastatic colorectal cancer treatment. Validating KRAS mutation profile on neuroimaging without histopathologic analysis would be beneficial. We aimed to differentiate Kirsten rat sarcoma (KRAS) gene mutation profile according to diffusion tensor imaging (DTI) metrics obtained from colorectal brain metastases (CRBM). Total of 8 patients with 17 CRBM included with known KRAS gene mutation status. All patients were categorized into two groups according to the KRAS mutation status as positive (+) (n: 5, 11 lesions) and negative (-) (wild type) (n: 3, 6 lesions). DTI values of the BMs were evaluated by consensus of two radiologists. Apparent diffusion coefficient (ADC), normalized apparent diffusion coefficient (nADC) and fractional anisotropy (FA) values of the two groups were compared. For comparison the Mann-Whitney U test was used. KRAS mutation (+) group showed significantly lower ADC and nADC values on CRBM rather than the wild-type. Comparison of the FA values of the two groups did not reveal any statistical significance. In conclusion, decreased ADC and nADC values of CRBM are correlated with presence of KRAS mutation. Lower ADC and nADC values obtained from CRBM can be utilized to insight KRAS mutation profile.

Keywords: Kirsten rat sarcoma gene mutation, diffusion tensor imaging, colorectal cancer, brain metastasis

Introduction

Colorectal cancer brain metastases (CRBM) are rare condition with an incidence ranging from 6% to 3.2% and brain involvement indicates a poor prognosis [1,2]. Detection of CRBM is increasing by advanced neuroimaging modalities, because of increased survival rates with new treatment options [3].

Metastatic colorectal cancer (CRC) treatment reached a new era with improvement of molecular-targeted drugs [4]. Molecular classification plays a significant role in treatment choice and estimation of the survey [5]. One of the key indicator is the Kirsten rat sarcoma (KRAS) gene that is shown in 30% to 50% of CRC. The presence of the KRAS mutation is associated with a worse prognosis and is resistant to treatment with epidermal growth factor receptor blockers in CRC with metastases [5-7]. Published data are limited regarding the molecular profile of CRBMs [8, 9].

Generally, biopsy or surgery are necessary to establish the KRAS mutation status; nonetheless, histopathological analysis may not sufficiently represent the exact KRAS mutation status of the entire mass because of tumoral heterogeneity, insufficient tumor tissue or inadequate sampling. Chemo-radiotherapy prior to the surgery negatively affects the histopathological evaluation. Therefore, it would be valuable to have a practical and non-invasive biomarker such as radiological imaging to reveal the KRAS mutation.

Brain MRI is the first method for diagnosis and following of CRBM. Diffusion tensor imaging (DTI), an advanced MRI method commonly used to estimate the structural integrity of white matter tracts, reveals changes at the microstructural level to assess tissue damage [10-12]. On the other hand, apparent diffusion coefficient (ADC) and fractional anisotropy (FA) are widely preferred DTI parameters for analyzing and characterizing primary and metastatic brain tumors.

DWI gives functional data depend on the random movement of water molecules. Highly cellular tumors diminish extracellular space and induce restricted diffusion, that is seen as hypointense on ADC maps and hyperintense on DWI.

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Recently, studies aimed to predict tumor characterization, aggressiveness, prognosis and treatment response with DWI are very popular. As known, internal genetic regulation of a tumor associates with its aggressive bio-behavior, prognosis and treatment options. Therefore, CRBM with different KRAS mutation status may affect the DTI features.

To our knowledge, there are no studies reporting the relationship between the KRAS mutation profile and the DTI features obtained from CRBM. We aimed to differentiate KRAS gene mutation profile according to diffusion tensor imaging metrics obtained from CRBM.

Materials and Methods

Patients

Our retrospective study involves the evaluation of Brain MRI of the patients with CRBMs in a period of January 2016 to December 2019. Selection criteria were circumscribed as biopsy confirmed CRC, reporting the KRAS mutation profile in the medical records of the medical oncology department and having brain metastases (BM). Exclusion criteria were set as artifacts in DTI sequences ($n=1$), small lesions (< 10 mm) ($n=1$) and MRI from outside institutions ($n=2$) (figure 1). As a result, 8 patients with 17 BM were included in the current research. Our university institutional ethical committee approved the study protocol (decision number: 08/145 date: 06.2020).

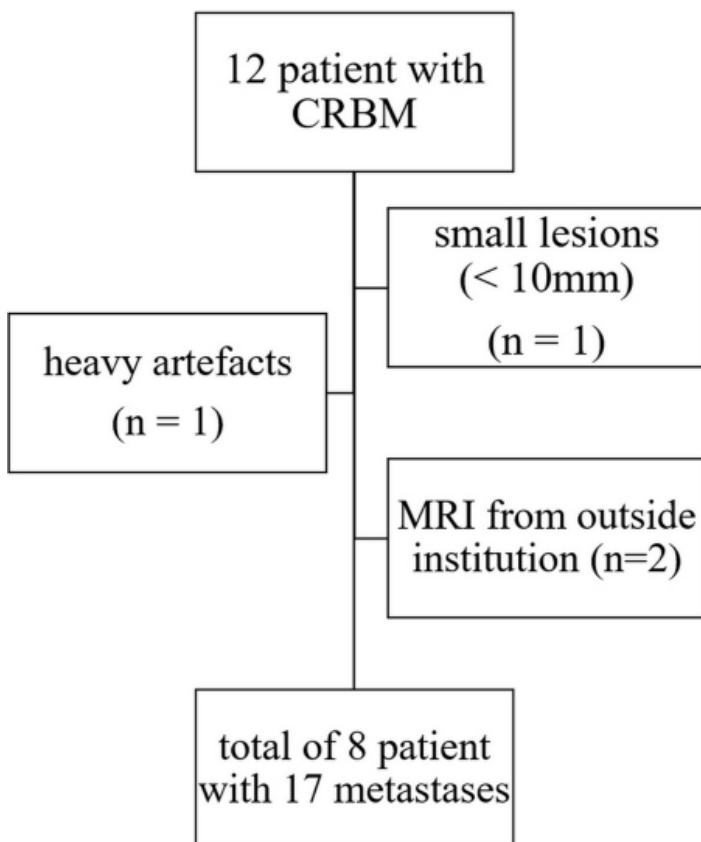


Figure 1. Diagram showing the exclusion criteria of patients for study
CRBM: Colorectal brain metastasis

MRI technique

MRI (1.5-T system Siemens, Avanto, Erlangen, Germany) study

was conducted using a maximum gradient strength of 43 mT/m and an 18-channel head coil. Routine brain MRI protocol (T1 weighted (T1W; repetition time (TR)/echo time (TE) = 475/10 ms) and T2W (TR/TE = 4870/87 ms) sequences in the axial and sagittal sections and fluid-attenuated inversion recovery (FLAIR) images (TR/TE/inversion time (TI) = 9000/87/2500 ms) in the axial and coronal sections with 5-mm-thick slices) was used for all patients. And then, T1W 3D magnetization prepared rapid gradient echo (3D-MPRAGE) (TR/TE/TI = 12.5/5/450 ms) sequences (with intravenous Gadolinium-diethylenetriamine pentaacetic acid, 0.1 mmol/kg body weight) were used. DTI protocol contained a single-shot, spin-echo, echo-planar sequence: TR/TE = 2900/85 ms; 128×128 matrix, 230 mm field of view and 5 mm slice thickness and 30 diffusion-encoding directions were used at $b = 0$ s/mm² and $b = 1000$ s/mm².

Image analysis

For post-processing of DTI data Leonardo console (version 2.0, Siemens) was used, ADC maps and colored FA maps were reconstructed before measurements. DTI values of the BMs were assessed by two radiologists in consensus (MAG and DHC) who were unaware of the KRAS mutation situation. DWI, T2W, FLAIR and contrast-enhanced 3D-MPRAGE T1W images were utilized to identify enhancing parts of the metastasis. Region of interests (ROIs) were drawn on the three distinct enhanced parts of the BM on ADC maps with avoiding cystic and necrotic portions of the lesions. Then, the eventual ADC value was considered as the average of the 3 different ROI measurements (figure 2). In order to justly analyze the distinctions between the ADC values of the metastatic lesions, the normalized apparent diffusion coefficient (nADC) ratio was calculated. To estimate the nADC values, the ADC value of the tumor was divided by the ADC value of the normal appearing contralateral white matter. The size of the ROIs ranged from 10 mm² to 35 mm² depending on the size of the metastasis.

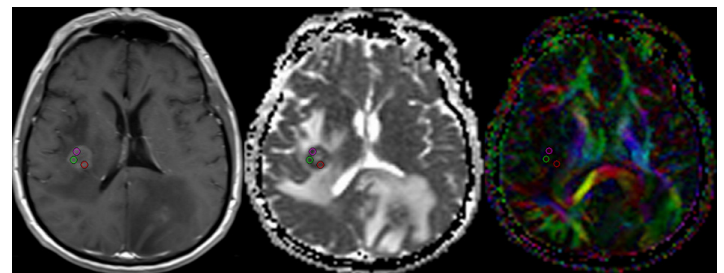


Figure 2. 51-year-old female patient with KRAS (+) CRBM. Contrast enhanced T1W, apparent diffusion coefficient and fractional anisotropy image. Images are showing the manual placement of the ROI within solid-enhancing metastatic tumor on three different localization

Histopathologic analysis

Pathological examination of KRAS gene mutation status was performed from primary colorectal tumor specimens. Exon 2 at codon 12 and 13, exon 3 at codon 59 and 61 and exon 4 at codon 117 and 146 were augmented by PCR and KRAS mutation status was analyzed.

Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics

20.0 program. For normality the Kolmogorov-Smirnov test was performed. Mean $\pm$ standard deviations presented as descriptive statistics were obtained. In comparison of the ADC, nADC and FA values of CRBM in KRAS (+) and wild-type groups, independent samples t-test was performed. A p values below 0.05 were considered significant.

Results

KRAS mutation positivity were found in 5 (%62.5) patients (3 female, 2 male, with mean age 53.6 years, range 42-74 years) with 11 (64.7 %) BMs. Remaining 3 (%37.5) patients (1 female, 2 male, with mean age 32.7 years, range 56-70 years) with 6 (35.3 %) BMs were considered as wild-type. Basic clinical information of the patients are shown in table 1.

Table 1. Basic clinical information of colorectal cancer with brain metastases

Factors		
Patient age (mean, range)		57 (42-74)
Gender	Male, n (%)	4 (%50)
	Female, n (%)	4 (%50)
KRAS status	Positive, n (%)	5 (%62.5), 11 metastases
	Negative, n (%)	3 (%37.5), 6 metastases

There was no statistically significant difference between KRAS mutation (+) and wild-type group in FA values ($p=0.518$).

Among the KRAS positive and wild-type groups, ADC ($p=0.034$) and nADC ($p=0.008$) values showed statistical significance. KRAS (+) patients exhibited significantly lower ADC and nADC values. The ADC, nADC and FA values of the two groups are presented in table 2.

Table 2. Results of ADC, nADC and FA values associated with KRAS mutation status

	KRAS (+) group (n=11)	KRAS wild type group (n=6)	P value
FA (mean $\pm$ SD)	204.1 $\pm$ 95	236.3 $\pm$ 97.9	0.518
ADC x 10^{-6} mm ² /s (mean $\pm$ SD)	836.5 $\pm$ 114.5	1014.2 $\pm$ 203.7	0.034
nADC (mean $\pm$ SD)	0.97 $\pm$ 0.21	1.33 $\pm$ 0.27	0.008

Discussion

The positive KRAS mutation status indicates unfavorable response to treatment regimens targeting the epidermal growth factor receptor road. National Comprehensive Cancer Network guidelines recommend performing KRAS tests for patients with CRC-related metastasis [4,13,14]. Therefore, correct mutation profiling is very crucial for the prognostic and therapeutic purposes. There were many studies that sought different methods for assessment of KRAS profile with non-invasive interventions and overcame the pathological analysis disadvantages [13,15-19]. Validating mutation profiles on neuroimaging would be favorable. For this objective, MRI and ¹⁸F-fluorodeoxyglucose-positron emission tomography imaging methods were used with different techniques and from different localizations, especially from the primary colorectal tumor [15,17,18] and metastatic liver lesions [13,16,19].

KRAS mutation activates G-protein signal transduction roads and induces diffuse proliferation while inhibiting apoptosis [20,21]. Moreover, KRAS mutation may be associated with increased angiogenesis and higher vascularization. Hypervascular tumors are often more aggressive which prompts accelerated tumor growth and increased metastatic potential.

Xu et al. demonstrated that DWI metrics may estimate KRAS mutation profile in rectal cancer and presented lower ADC mean values in the KRAS (+) patients [15]. [$1.26 \pm 0.36 \times 10^{-3}$ mm²/s vs $1.43 \pm 0.22 \times 10^{-3}$ mm²/s, KRAS (+) and wild type group, respectively]. They argued that the compact tumor cell structure and hypervascularity results with low ADC values in the rectal cancer. Another recent study reported lower ADC and ADC mean values in KRAS (+) patients compared to the KRAS negative patients in metastatic liver lesions due to the CRC. In these two studies, they evaluated primary tumor sites and metastatic liver

lesions, respectively while we made our measurements from the metastatic brain lesions. Metastatic brain lesions demonstrate similar histologic features to the primary tumor tissue. Clinical importance of the ADC values have been demonstrated especially in prediction of prognosis and characterization of tumor biological behaviors [22,23]. In our study, measurements from the BM of KRAS (+) group, yielded lower ADC and nADC values. This was consistent with the molecular profile of the primary tumor. Based on our findings, higher cellularity and vascularity of the lesions, aggressive behavior and poor prognosis correlate with the KRAS mutation along with lower ADC and nADC values.

The relationship between tumor cellularity and FA values has not been clearly revealed yet, and there are controversial results in the literature. [24-26]. We detected lower FA values in KRAS (+) patients in our study, however it was not statistically significant.

Our study had some limitations, mainly that study findings are preliminary results regarding the small study population. Secondly, manual placing techniques of ROI, that may be associated with biased results. Manual ROI technique is a rather simple and efficient method that can be performed in daily routine rather than whole tumor analysis methods. However, there are some disadvantages to using ROI for ADC measurements, such as size and position variations. Third, the absence of histopathological analysis of the BM. Histological diagnoses were made by evaluation of colorectal biopsy samples. Histopathological analysis of the BM is often unnecessary and not practical.

Conclusion

As to our knowledge, our study is the first to utilize DTI features to establish the KRAS mutation profile in CRBM. Decreased ADC and nADC values of CRBM are related with presence of KRAS mutation. ADC and nADC values of the CRBM may be utilized for

KRAS mutation prediction and may be a guidance for clinicians in their treatment decisions. Future studies with extended number of patients with more data will be beneficial in differentiating CRBM according to the KRAS mutation status without histopathological diagnosis.

Conflict of interests

The authors declare that they have no competing interests.

Financial Disclosure

All authors declare no financial support.

Ethical approval

Local Ethics Committee of Bezmialem Vakif University approved the study (decision number: 08/145 date: 06.2020).

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