Patterns and the Occurrence of KRAS Mutations in Metastatic Colorectal Cancers—a Study from Indian Regional Cancer Centre

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Abstract Mutation analysis of the KRAS oncogene is established as a predictive biomarker in Colorectal cancer (CRC). Many prospective clinical trials have shown that only CRCs with wild-type KRAS respond to anti-epidermal growth factor receptor (EGFR) treatment. Hence, mutation analysis is mandatory before treatment of metastatic CRCs. There are very few studies on the KRAS mutation status in the Indian setting. Hence, this study was done to document the patterns of KRAS mutations in CRCs reporting to a regional cancer centre in South India. Among 150 cases of metastatic colorectal cancer reporting over a period of 20 months, 48 random cases were analyzed for the KRAS mutational status of codons 12 and 13 of the KRAS gene by genomic

sequencing. KRAS mutations in codons 12 and 13 were present in 9/48 (18.75%) of all analyzed CRCs. The common types of mutations were glycine to aspartate on codon 12 (p.G12D), glycine to valine on codon 12 (p.G12V), and glycine to aspartate on codon 13 (p.G13D).

Keywords KRAS · Mutation · Metastasis · Colorectal cancer

Introduction

The Kirsten Ras (KRAS) is a member of the *ras* gene family (H, K, and N ras) and encodes highly similar membrane-localized G proteins with molecular weights of 21 kDa [1]. The KRAS proto-oncogene encodes for a guanosine triphosphate (GTP)/guanosine diphosphate-binding protein downstream of the epidermal growth factor receptor (EGFR) in the RAS/RAF/MAPK pathway [2]. Members of the *ras* gene family have been recognized as key targets in carcinogenesis due to their participation in controlling multiple pathways affecting cell growth, differentiation, and apoptosis by interacting with a series of coordinators and effectors as an essential component of the EGFR signaling cascade [3]. KRAS is involved in the pathogenesis of many different malignant tumors, including lung cancer, pancreatic cancer, and colon cancer [4, 5]. KRAS can acquire activating mutations in exon 2, codons 12 and 13 [6]. The prevalence of KRAS mutations varies among different human tumors. Earlier studies have shown the frequency of mutation to be around 30–40% in CRC and are similar across various ethnic groups [5, 7, 8].

The most frequent alterations are detected in codon 12 and codon 13, approximately 82 and 17%, respectively, of all reported KRAS mutations [9]. Mutations in other positions, such as codons 61 and 146, have also been reported. These alterations account for a minor proportion (1–4%) of KRAS

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mutations, and their clinical relevance in CRC is unclear [2–7].

It is important to identify the status of KRAS in each patient in order to plan the best therapy. Patients with the wild type (WT) can receive monoclonal antibodies against EGFR, while KRAS-mutated patients have no response to EGFR-targeted therapies and poor prognosis as seen in different studies [10–13]. There are very few studies on the KRAS mutation status in the Indian setting. The objective of this study was to assess the KRAS mutation patterns in primary and/or metastatic colorectal cancers reporting to a regional cancer centre in South India.

Materials and Methods:

This was a prospective study done at the Kidwai Memorial Institute of Oncology, Bangalore, a tertiary care oncology centre in South India. Eligible participants included males and females diagnosed with metastatic CRC between June 2013 and January 2015 who at the time of diagnosis, were aged 20–80 years. Patients reporting having received previous radiotherapy for CRC or other malignancy within 5 years were excluded. Among 150 subjects of metastatic colorectal cancer diagnosed during the above mentioned period, 48 subjects were randomly selected and analyzed for the KRAS mutational status of codons 12 and 13 of the KRAS gene by genomic sequencing. This number was limited based on financial and resource constraints, since this test was not routinely done as standard of care at the time of the study.

Assay to Detect Mutant KRAS DNA Extraction

The DNA samples were obtained from macroscopically dissected formalin-fixed paraffin-embedded (FFPE) specimens cut into 10- μ m thick sections or image-guided trucut tissue biopsy. The slides from FFPE sample were deparaffinized in xylene, washed in ethanol, and rehydrated. Any tissue surrounding the tumor was carefully pared away using scalpel under microscopic observation. The purpose of paring was to ensure that tumor cells comprised over 70% of the remaining specimen. After suspension in 400 μ l of 100-mMTris-EDTA buffer and proteinase K, the specimens were incubated for 3 days at 60 °C. At the end of incubation, the genomic DNA was extracted using commercial kits (DNA Blood Mini Kit, Qiagen) following the manufacturer's protocol.

PCR Amplification and Sequencing

In all cases, direct sequencing was performed using an Applied Biosystems 3730XL sequencer (PE Applied Biosystems) according to the manufacturer's instructions on PCR products purified using a Qiagen gel extraction kit.

Results

A total of 48 subjects with mCRC were studied. The baseline characteristics of the subjects are summarized in Table 1. The primary tumor location was the right colon in ten (20.83%), and the left colon in 38 (79.16%) of subjects. Of the 48 subjects, nine (18.75%) had the KRAS mutations in either codons 12 or 13. The distribution of mutations was 66.6% (6 subjects) in codon 12 and 33.3% (3 subjects) in codon 13. The common types of mutations were glycine to aspartate on codon 12 (p.G12D), glycine to valine on codon 12 (p.G12 V), and glycine to aspartate on codon 13 (p.G13D). Only five (10.41%) subjects of the 150 with CRC were treated with anti-EGFR therapy.

Discussion

In this study, we describe the results of routine KRAS mutation analysis in a sample of randomly selected metastatic CRCs, which shows a KRAS mutation frequency of 18.75%. In Western studies, the frequency has been found to range from 30 to 54%. Most of the studies have evaluated small numbers of selected CRC patients like the present study but have not specified the type of mutation [3, 14]. However, two other studies done in India estimate the prevalence of this mutation to be lower than that in Western studies [7, 15].

Routine KRAS mutation testing in metastatic CRC is increasing worldwide as the testing is a standard for metastatic CRC to evaluate the options of anti-EGFR-targeted treatment [14]. Many studies indicate that KRAS and other mutated molecules may be important prognostic factors related to disease-free survival and OS in CRC [13, 16]. The adverse prognosis of KRAS mutation on recurrence was observed in the Quick and Simple and Reliable (QUASAR) trial, which evaluated 5-

Table 1 Demographic characteristics

Patient characteristics	Present study
Number of subjects	48
Age in years—median	43 (22–65)
Gender, male: female	1:2.2
Primary site	
• Right colon	10 (20.83%)
• Left colon	38 (79.16%)
KRAS	
• Wild type	39 (81.25%)
• Mutated	9 (18.75%)
Mutation present	
• Codon 12	66.6% (6)
• Codon 13	33.3% (3)
Number of patients received anti EGFR therapy	5 (10.41%)

FU-based adjuvant treatment [17]. Samowitz et al. found the *KRAS* G13D mutation was associated with higher risk of death in a population-based cohort [16]. The characterization of *KRAS* mutational status in the chemotherapy alone subgroup of patients in the CRYSTAL and OPUS trials showed that the G13D mutation is associated with worse survival [18]. We need to further follow-up this patient population for an accurate survival analysis to document the outcome of such a mutation in the Indian setting.

Conclusion

In conclusion, *KRAS* mutation occurred in 18.75% subjects in this study. The common mutation patterns of *KRAS* seen are in exon 12. Studies involving more patients to validate these findings are needed in the future and in the Indian setting.

Author contributions SCS: Concept and design of the study, acquisition of data, writing the draft, revising the article, final approval of the article to be published

SBMC: Revising the article, final approval of the article to be published

LJA: Revising the article, final approval of the article to be published

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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