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RESEARCH ARTICLE

KRAS Mutation Status in Colorectal Epithelial Tumors of Colorectal Cancer

Amit Kumar Chakraborty[1], Soumit Dey[2], Ishita Ghatak[2], Pranamita Ray[2], Subhasree Bhattacharya[3], Archana Sengupta[4]

[1]Department of Genetics, Vivekananda Institute of Medical Sciences (VIMS), Kolkata, India. [2]Department of Pathology, Vivekananda Institute of Medical Sciences, VIMS, Kolkata, India. [3]Department of Gastroenterology, Vivekananda Institute of Medical Sciences, VIMS, Kolkata, India. [4]Department of Biochemistry, Vivekananda Institute of Medical Sciences, VIMS, Kolkata, India.

Abstract

Introduction: Colorectal cancer (CRC) is one of the commonest malignancies in industrialized nations with lower incidence in Asia and Africa. Majority of CRCs are non-hypermutated. KRAS is one of the commonest mutated genes in CRC patients. Given the KRAS mutated CRCs are resistant to EGFR targeted chemotherapy, expanded KRAS mutation testing is recommended before starting chemotherapy. The aim of this study was to study the genetic variation of KRAS in group of CRC cancer patients referred to a teaching hospital in Kolkata, India. **Methods:** Out of 15 cases of colorectal cancer studied, 2 specimens were right hemicolectomies for clinically diagnosed colon carcinomas and 13 specimens were biopsies of colonoscopy done for anaemia, changed bowel habit, occult bleeding. Formalin fixed paraffin embedded (FFPE) tissue sections, stained by routine Hematoxylin and Eosin were examined microscopically. The DNA was extracted from FFPE tissue with Qiagen kit. KRAS Mutation analysis was done by TheraScreen – KRAS Mutation Kit. **Results:** The age of patients ranged from 28 to 64 years. Nine were male and remaining 6 were female. histopathological examination revealed 10 adenomas and 5 adenocarcinomas. One adenoma, tubulovillous with high grade dysplasia featured positive KRAS mutation (Gly12Asp). Three out of 4 adenocarcinomas with positive KRAS mutation, one showed Gly12Ala and 2 showed Gly12Asp. **Conclusion:** The frequency of KRAS mutations was very low in the studied sample.

Keywords: Mutation. KRAS. Adenocarcinoma- Dysplasia- Epithelial neoplasm

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Introduction

Colorectal cancer (CRC) is one of the commonest malignancies in industrialized nations [1]. The incidence is much lower in Asia and Africa. The striking difference in incidence across geographic regions may be largely contributed by diet. For instance, the high consumption of meats with animal fat has been seen to be associated significantly with this neoplasm.

CRC shows reportedly a low incidence in India and also variable presentation across regions [2]. Mortality is higher in countries with limited health related resources. It have been decreasing in many Western countries due to several factors including early detection of the pathology by effective screening and improved treatment of CRC [3].

In colorectal cancer age variation is somehow different. A study by Patil PS et al, showed 80% of patients were below 60 years. In other 2 studies the mean age of presentation was 47.01 years and 58.4 years respectively [4, 5]. Another study from central India reported the median age at diagnosis to be 43 years with 39% of CRC cases being diagnosed at the age of 40 or less [6]. Whereas peak incidence is in 7th decade in United States [1]. The commonest sites affected are sigmoid colon and rectum.

Inherited predispositions are common in CRC patients presenting at younger age. Most common is Lynch syndrome- with germline mutations in DNA mismatch

Corresponding Author:
Dr. Ishita Ghatak
Department of Pathology, Vivekananda Institute of Medical Sciences, VIMS, Kolkata, India.
Email: ishitaghatak80@gmail.com

repair genes- most commonly MLH1, MSH 2 and at times PMS2, MSH6, EPCAM. About 15% sporadic CRC also show dysfunction of DNA mismatch repair due to methylation of MLH 1 promoter [7]. The neoplasm is more common in proximal colon in this context. Other inherited syndromes are FAP, MUTYH associated polyposis, serrated polyposis, Hereditary mixed polyposis syndrome, Peutz Jeghar syndrome, juvenile polyposis and Cowden syndrome [8]. Ulcerative colitis and Crohn disease are two chronic inflammatory bowel diseases associated with significantly increased risk of developing colon cancer.

Majority of colorectal carcinomas are non hypermutated type [1, 9]. Most commonly mutated genes in this group includes APC, TP53, KRAS, PIK3CA, and SMAD4. KRAS mutation presents in 40-50% of CRCs. Another 10-20% CRC cases have BRAF or NRAS mutations. These are downstream proteins in the epidermal growth factor receptor pathways [10, 11]. CRCs with RAS mutations are resistant to EGFR targeted chemotherapy . Hence expanded RAS mutation testing is recommended before starting chemotherapy in colon cancer.

This study was conducted in a tertiary care teaching hospital in Kolkata. It studied a group of 15 cases of colonic epithelial neoplasms. The aim was to observe the occurrence of genetic variations in KRAS gene.

Materials and Methods

Fifteen cases of colonic epithelial neoplasms were selected for the study. The age ranged from 28 to 64 years. Nine were male and remaining 6 were female. The specimens were received and processed in the histopathology department of the hospital. Two of those were right hemicolectomies for clinically diagnosed colon carcinomas. Remaining 13 cases were colonoscopic biopsies from mass or polyp in colon. The indications of colonoscopy in those cases were anemia, change in bowel habit, occult GI bleeding, etc.

History and demographic data were retrieved from request forms of specimens sent for histopathological examination (HPE), i.e. tissue diagnosis. The genetic analysis was performed in the Department of Genetics.

The specimens were processed and examined by light microscopy after routine Hematoxylin and Eosin staining of formalin fixed paraffin embedded (FFPE) tissue sections.

In this study the source of genetic material was FFPE tissue sections of thickness of 10 µm. The DNA extraction was done using a commercially available extraction kit Qiagen (QIAamp DNA FFPE Tissue Kit) using the instructions of the manufacturer. Tissue sections were deparaffinised with xylene followed by wash in ethanol. Tissue lysis was done by Proteinase K at 56˚C and the resultant mixture was loaded into the extraction column. DNA was eluted by elution buffer of 20 mL (by principle of affinity chromatography). The concentration and quality of extracted DNA were determined by nano spectrophotometer.

KRAS Mutation analysis was done by the ARMS/ Scorpion based TheraScreen – KRAS Mutation Kit{ KRAS RGQ PCR KIT(24)/QIAGEN }. This assay is devised to detect 7 most common mutations in KRAS gene , namely Gly 12 Ala, Gly12Asp, Gly 12 Arg, Gly 12 Ser, Gly 12 Cys, Gly 12 Val and Gly 13 Asp on exon 2. Each cases was evaluated for these 7 mutations by a real time quatitative PCR assay with Therascreen KRAS RGQ PCR KIT(Qiagen).

Results

Histopathological examination revealed 10 cases of adenoma and 5 cases of adenocarcinomas. 1of the adenomas was tubulovillous type with high grade dysplasia featuring positive KRAS mutation (Gly12Asp with base change of GGT>GAT). 3 of the 5 adenocarcinomas showed KRAS mutation. Of those 1 showed Gly 12 Ala (base change GGT> GCT) and 2 were positive for Gly12Asp (base change GGT> GAT). The age of the patients with positive mutations ranged from 53-55years. All mutations here were in codon 12.

Discussion

EGFR signal transduction pathway is a complex and finely regulated process associated with proliferation, growth and survival of normal cells. When this system is uncontrolled, it may lead to growth, proliferation, survival, and metastasis of neoplastic cells. Abnormalities within the EGFR signaling pathway , such as gene mutations or amplification, and protein overexpression, have been seen to be associated with colorectal carcinogenesis [12].

The KRAS is a proto-oncogene. It encodes a guanosine 5′-triphosphate- (GTP-) binding protein of 21-kDa molecular weight. The protein acts at the beginning of the MAPK signaling pathway. Somatic mutations in KRAS are observed in many cancers, including 30%–40% of colorectal cancers. These mutation is an early event in carcinogenesis [13-17]. Most commonly missense mutations occur in Codon 12/13. They result in constitutive activation of the KRAS protein with loss of GTPase activity. These mutations result in unregulated downstream signaling causing activation of various transcription factors leading to cell survival, proliferation, differentiation, migration. These neoplasms are not responsive to antibodies that target the EGFR receptor.

Belly RT, et al, [18] and Tejpar S and colleagues [19] show in their respective studies, the occurrence of shorter survival in CRC with KRAS mutations. However, in another study, Roth AD, Tejpar S, et al, [17] and Ogino S ,et al, in their trial [20] do not support this hypothesis. KRAS mutation status is a strong predictive marker of resistance to EGFR-targeted therapy in metastatic colorectal cancer (The mutations predict lack of response to anti-EGFR monoclonal antibodies cetuximab and panitumumab) [21, 22].

In Bengaluru, Karnataka, Veldore VH et al, (2014) showed prevalence of KRAS mutation to be 42.8% in metastatic CRC. Ninety two percents of the mutations were present in Codon 12 and 8% were in Codon 13.

Glycine to Arginine substitution was seen predominantly in rectosigmoid followed by cecum, while Glycine to Alanine mutation was relatively more frequent in sigmoid, followed by rectum and rectosigmoid [23]. The researchers extracted genomic DNA from paraffin embedded tumor tissues. The DNA was screened for 7 point mutations in Codons 12 and 13 of KRAS gene, using Scorpions amplified refractory mutation system real time polymerase chain reaction technology as we observed in our study.

Jauhri M et al, in a study at New Delhi (24), showed the mutation frequency of KRAS to be 35.7% in Indian CRC patients. Mutations in Codons 12 and 13 together constituted the bulk (81.4%) of all KRAS mutations. All mutations were of missense type. The three most common mutations were substitution of glycine with aspartate (G12D) or valine at codon 12 (G12V) and substitution of glycine with aspartate at codon 13 (G13D). These three mutations occurred as a result of substitution at c.35G>A, c.35G>T, and c.38G>A. In this study, for molecular analysis , next -generation sequencing was performed to identify mutations in the six potential biomarker genes including KRAS using FFPE tissues.

The present study was conducted in Kolkata,West Bengal. Of the 15 cases, all 4 mutations were in codon 12, consistent with literature. 1 case showed Gly 12Ala substitution in adenocarcinoma in a large polyp in the descending colon. The adenocarcinoma showed mucinous morphology. The remaining all 3 cases showed Gly12Asp substitution- 1 of the 3 commonest mutations as shown by Jahuri M et al. Of those 3, one was an adenoma in sigmoid colon polyp. Morphology showed tubulovillous adenoma with high grade dysplasia. The other 2 were conventional adenocarcinomas in right sided colonic polyps in either. In other words, only 1 out of 11 adenomas(9%) was positive for KRAS mutation . Three out of 5 cases of adenocarcinoma (60%) showed KRAS mutation. These prevalence of mutation was not consistent with the prevalence rate of 35.7% to 42.8% as shown in literature [23, 24]. In this study, frequency of KRAS mutation in sporadic colonic adenoma and adenocarcinoma were not comparable to frequency in Indian population as shown by studies in other regions of India because of very limited sample size, differences in methods and difficulty in processing. For the same reason, pattern of mutations could not be studied. The genomic DNA was isolated from FFPE tumor tissue, which was difficult as nucleic acids formed strong bonds during tissue processing. Retrieval of DNA by cleaving those bonds caused loss of quality DNA material. Besides, Real time PCR by QIAGEN detected only 7 types of mutations. Hence mutations outside these 7 went undetected in this series. False negative cases were likely

The present study at Kolkata evaluated the morphology and KRAS mutation status of a series 15 cases of colonic epithelial tumors. The frequency of KRAS mutations was not comparable to the frequency in Indian CRC patients, as shown in researches in other regions of India, due to very small sample size, difficulties and limitations of the method and also differences in method with the reference studies. This was only a small attempt to observe the mutation status in the said context of colonic epithelial tumor. Nevertheless a more comprehensive prospective study with a large sample size and a more sensitive method to detect several variants of KRAS mutation in this region of India is needed to determine the prevalence and pattern of KRAS mutations and also its role as a determinant of prognosis.

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