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Incidence of KRAS mutations in colorectal carcinomas in a tertiary care centre

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Introduction

Colorectal cancer (CRC) is the third most common cancer and the second most common cause of adult cancer related deaths worldwide.[1] Carcinoma of the colon and rectum is a relatively uncommon malignancy in India when compared with the western world.

Colorectal cancer is generally a disease affecting individuals 50 years of age or older.[2,3,4] It has been estimated that between 2 and 3% of colorectal cancers occur in patients younger than the age of 40 years. Men have proportionately higher incidence of rectal cancer than women.[5,6,7]

Colorectal carcinomas (CRCs) evolve through multiple pathways. These pathways may be classified as two different subgroups, which are defined based on their molecular features: (1) chromosomal instability and (2) chromosomal stability. There are hereditary and sporadic tumors in both groups. The minority of hereditary tumors showing chromosomal instability are related to familiar adenomatous polypopsis(FAP) while tumors showing chromosomal stability are hereditary non-polypous colon cancers(HNPCC). Chromosomal instability is characterized by an increased rate of loss or gain of large portions of chromosomes or whole chromosomes. Approximately 85% of sporadic tumors showing chromosomal instability evolve through the classical adenoma-carcinoma sequence. The most frequent mutation in these tumors is the colorectal cancer gatekeeper gene, the APC gene mutation, which involves want signalling mediated by β-catenin.[8] It is always present in FAP patient as a germ line mutation involving one of the alleles. Somatic mutation, loss of heterozygosity (LOH) of the second allele causes the inactivation of the gene. However, APC inactivation is not required in all instances of neoplastic initiation and evolution of colorectal cancers. KRAS and p53 mutations are strongly associated with advanced adenomas. The serrated hyperplastic aberrant crypt foci usually have BRAF mutation, while their minimally serrated counterparts usually have KRAS mutation.[9] KRAS mutation is very strongly associated with a villous architecture[10] and both hyperplastic polyps and serrated adenomas show frequent mutation of the oncogene BRAF as well as extensive DNA methylation,[11] which is much more a feature of tumors showing chromosomal stability. In serrated adenomas without mutation of BRAF, the most frequent mutation is mutation of KRAS. The incidence of K-ras mutation was extremely low in this group of early cancers.[12]

According to multistep route of genetic alterations in the colorectal adenoma-carcinoma sequence, KRAS mutation is one of the first alterations to occur.[13] Activating mutations in the KRAS proto-oncogene gene are involved in 25 – 60% of colorectal carcinomas.[14] The importance of KRAS mutation testing for determination of eligibility to receive anti-EGFR therapy has been well established.[15,16]

Objectives

This study was carried out to find out the incidence of KRAS mutation in diagnosed cases of colorectal carcinomas.

Materials and Method

This is a three year study from February 2012 to February 2015 in a tertiary care centre. In this study, all patients diagnosed with colorectal carcinoma who undergo KRAS mutation analysis were included. Histopathological and molecular studies were done on formalin fixed paraffin embedded tissue blocks. The diagnosed cases of colorectal carcinomas include biopsies, resection specimens and review slides / blocks. From the formalin fixed paraffin embedded blocks, slides were prepared and stained with haematoxylin and eosin stain.

KRAS mutation analysis was done by real time polymerase chain reaction (PCR) method on the Rotor-Gene Q instrument PHoTOS of PCR SET UP.

After the pathological diagnosis, the block containing a minimum of 40% tumor tissue was chosen for KRAS mutation analysis. 2x20micron curls were taken on a microtome into a microcentrifuge tube. Further the tumor genomic DNA was isolated using QIAAMP DNA mini kit (15704) by using standard procedures as per manufacturer recommendations.

- PHOTOS OF CRC. CRC labelled as moderately differentiated colorectal carcinoma (H&E 10x)
- CRC 1 labelled as moderately differentiated colorectal carcinoma (H&E 40x)

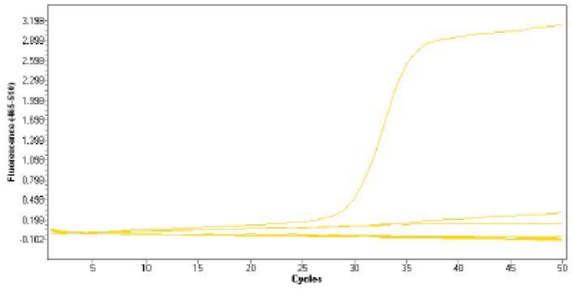

Fig. 1: PCR KRAS wild type graph reading

The quality and the quantity of the DNA was verified by nanodrop spectrophotometer and further used for molecular screening. There were seven known somatic point mutations which are most commonly reported in colonic tumors. The Therascreen KRAS mutation detection kit, has primers and probes to accurately detect all the seven point mutations at a specificity of 100% and sensitivity upto the level of less than 1%.

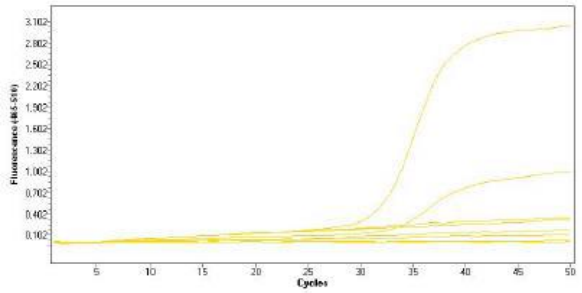

Fig. 2: PCR KRAS mutation graph reading

The PCR reaction was setup as per the instruction protocol and the sample was processed on LC480 platform from Roche. The mutation profiles were assessed based on the kit literature for determining the mutation status.

The therascreen kit adopts scorpions arms technology which has very high specificity and good sensitivity which is met with the Scorpion arms technology. 25ul reaction where in 50ng of nanodrop quantified DNA was added to mastermix composed of PCR reagents. The results are correlated with other clinical finding in each of these cases.

Results

260 patients diagnosed with colorectal carcinoma over age group of 15 to 75 years who underwent KRAS mutation analysis were included in this study. A morphological and molecular study was done.

The incidence of KRAS mutation was calculated, incidence was found around 58% in study population the rest 42% were wild type mutations. The maximum number of cases with KRAS mutation was seen in the age group of more than 60 years. Females outnumbered the males. The youngest and the oldest ages recorded were 15 years and 72 years respectively.

Discussion

The frequency of mutations in the KRAS oncogene has been reported to vary between 25 and 60%.[14] This broad range of reported frequencies of KRAS mutations may be due to various factors such as sensitivity and specificity of mutation detection methods, small series of selected patients and variability in analysed gene region. In this study the analysis of KRAS mutation is based on a highly sensitive and specific detection method and incidence was found to be 58%.

Although it is now accepted that determination of KRAS status should play an important role in determining the utility of anti-EGFR therapy, it is less clear how KRAS status should be determined. A number of tests for KRAS mutations are available, the majority of which are laboratory based.[17] Direct sequencing analysis and real-time PCR are commonly used tests for detecting KRAS mutations.[18] Direct sequencing analysis is capable of detecting all possible mutations in exons 2 and 3 of KRAS but may lack sensitivity compared with other methods. Real-time PCR uses oligonucleotide primers that bind specifically to the most common in mutations codons 12 and 13 and, although highly sensitive as a result of preferential amplification of the mutant allele in large excess of the wild-type allele, will identify only those mutations targeted. Although it is possible to detect uncommon mutations, tests that screen for rare mutations are generally unavailable. The TheraScreen KRAS mutation assay is an allele-specific PCR assay that detects 7 common mutations in codons 12 and 13 of exon 2 for patients who are candidates for panitumumab or cetuximab therapy.

The clinical implication and importance of KRAS mutation in colorectal carcinomas is in giving the anti-EGFR therapy to the required patients. Patients with mutated KRAS colorectal carcinomas are unlikely to benefit from anti-EGFR therapy, it remains unclear that with KRAS wild type colorectal carcinomas will definitely respond, although these individuals may be able to derive some benefit from anti-EGFR therapy. Patients with metastatic colorectal carcinomas are being considered for anti-EGFR antibody therapy should be tested for the presence of a KRAS mutation prior to therapy.[19] Cetuximab[20] and Panitumumab[21] have a favourable survival impact in patients with KRAS wild type colorectal carcinomas, both agents should be initiated only in patients with KRAS wild type colorectal carcinomas.

Conclusion

KRAS mutations in colorectal carcinomas occurs usually in elderly age group with female preponderance. Incidence of KRAS mutations in colorectal carcinomas plays a very important role in determining the anti-EGFR therapy to the patients. Assessment of KRAS mutational status will likely

become a routine aspect of analysis of colorectal cancer, similar to routine HER2 protein analysis by immunohistochemistry or HER2 gene analysis by in situ hybridization in breast cancer. As with HER2/HER2 analysis,[22] there is a need for rigorous application of best practices to ensure accurate assessments of KRAS status. Pathologists verify the presence of tumor material in the tissue section or block to be used for DNA extraction and assess whether there is sufficient quantity and quality of tumor material for KRAS testing[18] this is an especially important function given the observation that KRAS mutations are visible early in tumorigenesis.[2.23-25] Pathologists also determine whether the percentage of tumor tissue in the selected tissue section or block is above the lower limit of quantitation of the selected KRAS test used in the laboratory. In addition, pathologists evaluate and select a molecular diagnostic laboratory for KRAS testing, select the appropriate technology for diagnostic laboratories, and guide oncologists and team members in the interpretation of results.[18]

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