

REVIEW ARTICLE

Kras Diagnosing the Little-Known Cancers Oncogene through Liquid Biopsy: Review

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ABSTRACT

Tissue biopsy, till date, is a gold standard for tumor diagnosis, grading, treatment, and detecting genetic evidences for identifying appropriate personalized treatments. However, it is painful, invasive, expensive, and risky making sequential biopsies basically impractical. Detection of Kras genes through liquid biopsy is the growing theragnostic technique, which is more sensitive, specific, much cost-effective and quick method for detecting the mutational status of cancers. Liquid biopsy detects biomarkers present in various body fluids, such as plasma, urine, saliva and cerebrospinal fluid, harboring cancer degraded fragments and cells shed by carcinoma such as circulating tumor cells, microRNA and circulating tumor DNA. It can be utilized as a pre-screening test for initial stage cancers also where multiple sampling is required for monitoring cancer therapies. Kras is the most extensively mutated cancer oncogene involve in altering the downstream signaling pathways, increasing oncogenic signaling, which is typically associated with poor prognosis and resistance to therapy. This review was conducted to clarify its prognostic significance as well as its mutational role in different carcinomas. To identify studies related to Kras mutation Medline, PubMed, Google Scholar and Web of Science search engines were explored and forty two relevant researches were finalized from year 2005 to 2019.

Keywords: Kras Gene; CfDNA; Liquid Biopsy; CtDNA.

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INTRODUCTION

The growing theragnostic requirement for the management of malignancies have led to more sensitive, specific, much cost-effective and quick methods for the detection of mutational status of Kras in different cancers¹. Although tissue biopsy is still the gold standard for tumor diagnosis, grading and detecting genetic evidences, yet it is painful, invasive, expensive, and risky especially for sequential biopsies. These lead to undue delays in treatments of fast growing malignancies where time matters seriously².

Liquid biopsy, for initial tumor identification, detects blood based biomarkers which are cancer degraded material and cells shed by carcinoma i.e. ctDNA, CTCs, exosomes etc. They can also be detected in various body fluids, such as urine, saliva and cerebrospinal fluid³. The oncogene family

of Rat sarcoma (Ras) is made up of Kras, Nras and Hras⁴. These genes encode 21kD monomeric GTPases that are involved in transmitting signals from extracellular to intracellular signal transduction. However, Kras has significantly higher mutational status in different cancers than Hras and Nras. Thus, in the present review, we investigated the role of Kras mutation in diagnosing different cancers through liquid biopsy (cfDNA).

DISCUSSION

Tissue biopsy, till date, is a gold standard for tumor diagnosis, grading, staging and detecting genetic evidences for identifying appropriate personalized treatments. On the other hand, it is not possible when patient is in critical condition or tumor is inaccessible or too invasive. In addition, tumor heterogeneity can prevent accurate genotyping of the tumor samples leading the clinician's clueless. In

emerging countries, like Pakistan there are a lot of inherent difficulties related to performing tissue biopsies like potential surgical complications, clinical risks, discomfort and above all the medical expenditures².

Detection of circulating tumor DNA (ctDNA) through liquid biopsy is a gold mine for detecting mutations and possesses the potential of devising future personalized treatments as it targets the DNA of degraded cancer cells which is released in blood circulation and in other body fluids like urine, CSF. These degraded cancer cells are source of fragments of tumor DNA (ctDNA), exosomes, cell-free RNA (cfRNA) and circulating tumor cells (CTC)⁵. The ease of collection of these fluid samples enables one to do repeated liquid biopsies with less invasive, easily accessible, and in a much convenient fashion compared to conventional tissue biopsy. It also helps in monitoring tumor progression by taking multiple samples, which keep an eye on tracking mutations and response to treatment⁵.

Genomic biomarkers are recently being extensively investigated for both as predictive and prognostic tools for various malignancies. Presently, different variations have been detected in biomarkers, which are considered significant in different malignancies, including Pancreatic, Lung, Colorectal and Prostatic Carcinoma. Identifying allele-specific therapeutic approaches is the ultimate goal of researching human Kras mutations⁶. Over the past several decades, there has been extensive research in contrasting mutant and wild types (WT) of Ras oncoproteins with relatively slight attention given to potential variations between specific mutations resulting in the activation of oncoproteins⁷. Mutations in Ras proteins shift homeostatic balance to continuous active state either by reducing GTP hydrolysis or by increasing GTP loading level. Nucleotide binding specifies the activation state of the RAS proteins with an active signaling conformation of the GTP-bound form. This has only been observed for G12C, where inhibitors that can covalently bind to the cysteine and inhibit the activated oncoproteins.

Scientific evidence supports the idea that the genetic characteristics of each allele can be useful for various allele specific therapies. For instance, Kras testing for mutations for metastatic colorectal cancer (mCRC) has been recommended by the National Comprehensive Cancer Network (NCCN) guidelines as part of the preliminary diagnostics⁸.

This review offers perspectives from both clinical and observational research to examine the similarities and differences of Kras allele in various cancers.

Kras Allelic Variation

KRAS, a prominent member of the Ras family, plays a major role in healthy tissue signaling through cycling of GTPase between active GTP, Kras GTP and Kras GDP, which is inactive formations⁹. Mutations in KRAS can impair GTPase's function, leading to continuous activation of AKT / mTOR / PI3K and MEK / ERK / RAF, the downstream signaling pathways¹⁰. Multiple studies have documented that Kras mutation can boost cell proliferation; lead to malignant transformation¹¹. Thus, the ongoing activation of Kras would ultimately develop into malignancies. Numerous studies have shown that Kras mutation is a predicting as well as prognostic biomarker for cancer patients.

The ongoing research has not been able to evaluate the prognostic effect of cfDNA-detected Kras. This review discusses the wide range of evidence for Kras genes that are mutationally distinct. Among the most common cancers with Kras mutations are the pancreatic carcinoma, colorectal cancer (CRC), and non-small cell lung cancer (NSCLC). Mutations on codon no.12 are most prevalent and are a cause of almost 90% of all Kras mutation^{12,13}. Apparently, the likelihood of various missense changes in the absence of codon 12 depends on the type of cancer. In NSCLC, it is possible to explain the increased frequency of particular alleles derived from a classic smoking induced mutation (G:C to T:A) transversion¹⁴ which are the most common Kras mutations:

1. G12V (GGT to GTT)
2. G12C (GGT to TGT)

This pattern of mutational enrichment does not extend beyond NSCLC, and the predominance of common codon no. 12 alleles in various tumor contexts appears highly unlikely to be explained by mutational trends. In different codons, the mutation rate is unpredictable, but in some tumors, mutations in codon no.12 account for a significant proportion of Kras activating alleles, for instance, NSCLC and PDAC. Mutations in codons no. 13, 146, and 117 are more common in colorectal cancer. Following is an overview of the different types of cancers, which are a result of mutations in the Kras oncogene (Figure 1).

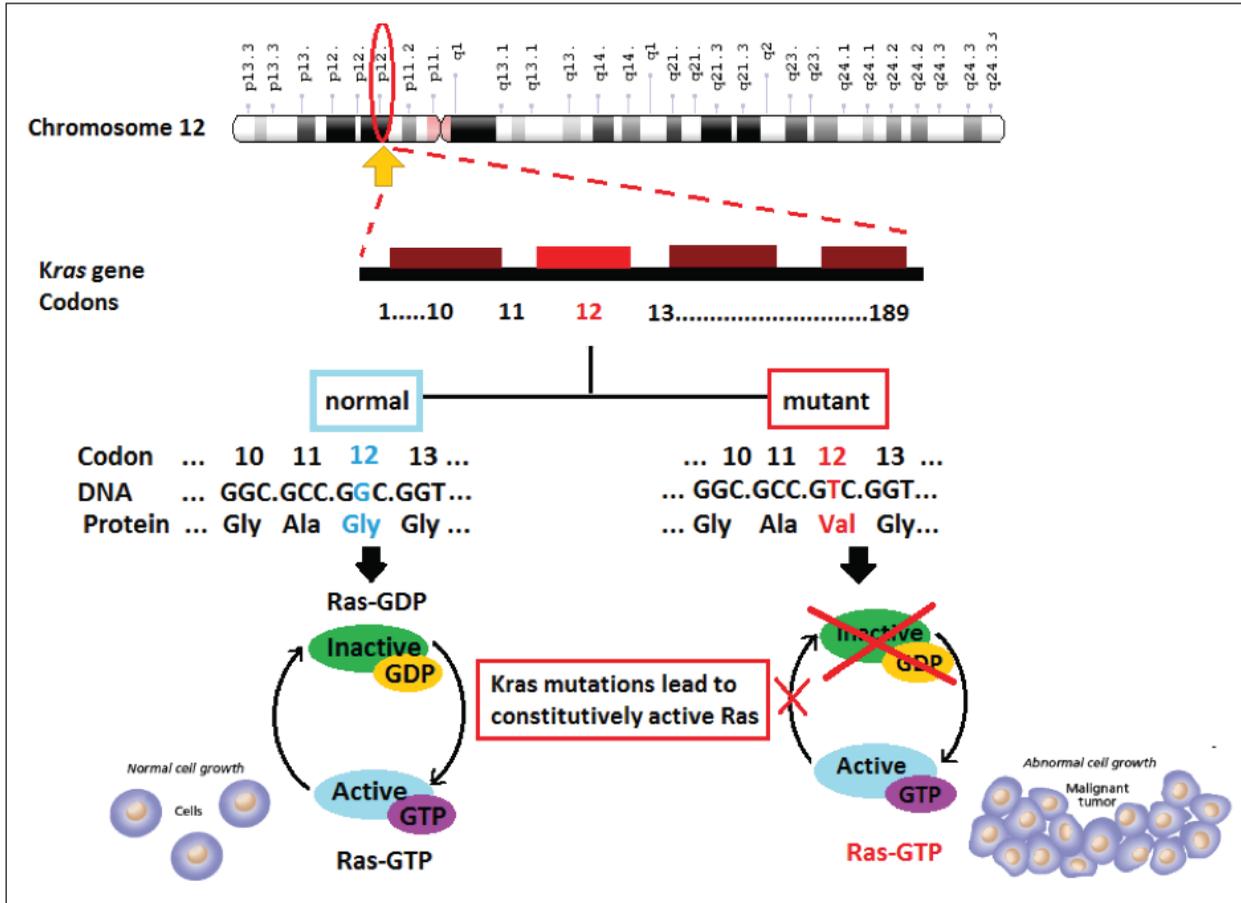


Figure 1: Kras gene present on chromosome no. 12. Normal (wild type) ‘Kras cycles between an active-GTP bound state and an inactive GDP-bound state, and it is largely in an inactive state in non- dividing cells’. Mutant Kras is continuously in an active GTP-bound state, results in abnormal cell growth and proliferation.

Pancreatic Ductal Adenocarcinoma (PDAC)

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive tumor with a survival rate of less than five years¹⁵ and an increasing occurrence around the world¹⁶. Poor prognosis depends on numerous factors, which include late diagnosis, drug resistance and drug targets ineffective to improve patient condition¹⁷. Most of the time it is difficult to resect pancreatic tumor (tissue biopsy) and the only validated circulating blood based biomarker in the National Comprehensive Cancer Network (NCCN) guidelines for routine clinical management is carbohydrate antigen 19-9 (CA 19-9), which is hindered by sub-optimal sensitivity and specificity. In recent times, liquid biopsy has been identified as a minimally invasive substitute for conventional blood-based biomarkers as well as invasive tissue biopsies for different types of cancers, including PDAC^{18,19}.

The most prevalent oncogene, which is mutated in pancreatic tumor, is Kras oncogene²⁰, and in 90 percent of PDAC cases, such mutations were observed²¹. The most widespread Kras mutations occur in codon 12, namely G12D, G12V, and G12R,

with 51%, 30%, and 12% respectively²². Although, the presence of Kras mutation has been reported to be correlated with a reduction in overall survival²³. There is limited use of Kras mutations as a prognostic marker, as only about 20% of patients with a resectable tumor is present. In this context, a prognostic, noninvasive blood test for PDAC would be very valuable. A recent study stated that mutations of Kras gene were found in 96.1 percent of conventional tissue biopsies, while 80 patients (80.5 percent) had Kras mutations in cfDNA with a median conc. of 0.165 copies/ Litre and a median fractional abundance of 0.415%²⁴. Another research showed that 58.9% of PDAC patients harbor Kras mutation, along with metastasis and patients with locally progressive disease (18.2%), through liquid biopsy²⁵. In another study, “measurable copy number alterations (CNA)” were observed through cfDNA samples of 55 patients (9 metastatic specimens, 1 locally advanced), seven of which showed a gain in 12p chromosome harboring Kras. Kras also revealed “non-synonymous somatic mutations” in all seven pancreatic ductal adenocarcinoma cfDNA samples with copy number gain²⁶.

Non-Small Cell Lung Cancer (NSCLC)

Globally, lung cancers are the leading cause of death due to malignancies. More than 80 percent of all lung malignancies, non-small cell lung cancer (NSCLC) are the most common type. However, multiple oncogenic driver variations have been identified in the last decade, each of which is a promising therapeutic target. In lung adenocarcinoma patients, Kras mutations are the most common oncogenic alterations, for which effective therapies have not yet been developed²⁷.

A variety of genetic aberrations have also been reported in NSCLC over the past decade, including “Kristen Rat Sarcoma viral oncogene (Kras), Epidermal Growth Factor Receptor (EGFR) and Anaplastic Lymphoma Kinase (ALK)”, the most frequently transformed oncogenes that act as genomic tumor drivers¹³. As far as Kras oncogene is concerned, although the “KRAS-MAPK pathway” is downstream signaling of EGFR, adenocarcinomas, with Kras mutation do not respond to EGFR tyrosine kinase inhibitors²⁸. A meta-analysis of 28 studies was conducted, in which 3620 Kras mutated lung adenocarcinomas patients showed poor prognosis, but not in histology of squamous cell carcinoma²⁹. Retrospective studies showed that patients with Kras mutations (G12C) in early stage or advanced NSCLC have significantly shorter overall survival compared to other Kras mutations³⁰.

Colorectal Cancer

Globally, colorectal carcinoma (CRC) is one of the most common malignancies, stands second in males and third in females causing fourth and third place mortality among men and women respectively³¹. Across the world, CRC affects over one million men and women per year and is cause of half a million deaths³². Genetic mutations,

advanced age, gender, lifestyle modifications, inflammatory bowel disease, positive family history along with previous history of hemorrhoids are the risk factors associated with incidence of CRC³³.

In CRC, the presence of multiple genetic aberrations such as “Kras, Braf, and PIK3CA” has been reported. “The Kras proto-oncogene encodes a guanosine triphosphate (GTP)/guanosine diphosphate (GDP) binding protein that acts downstream of epidermal growth factor receptor (EGFR) in the RAS/ RAF/MAPK pathway”³⁴. The presence of mutated Kras gene i.e. 35–40% is an early phenomenon in colorectal cancer patients³⁵. Another study conducted on CRC patients revealed 50% Kras mutation and 10% BRAF mutations³⁶. Most of the mutations, which occur in Kras gene, are oncodons 12 and 13 of exon 2 (90%), and these mutations are recognized as predictive biomarkers for the treatment of metastatic CRC patients with anti-EGFR drugs³⁷. The Sanger Cosmic database (n=34,958), determined 35% of CRC patients harbor Kras hotspot exon 2 mutations³⁸.

Presently, the drugs used are directed towards the ‘epidermal growth factor receptor’ (EGFR) for the treatment of CRC is determined because of the mutation status of the Kras and Braf genes. Therefore, tests to detect mutations in these genes are made before targeted therapy for colorectal cancer is decided³⁹. However, the presence of mutational Kras status negatively affects the efficacy of therapies that inhibit the EGFR⁴⁰. Kimura T et al. stated that by knowing the Kras mutational status it could be helpful in identifying patients who will negatively response to anti- EGFR treatment⁴¹. Therefore, earlier detection of mutated Kras gene plays an important role in the prognosis of CRC patients. Thus, the methodological aspects of the Kras tests and the types of assays are important⁴².

Table 1: Studies showing the role of ctDNA in detection of Kras mutation in different cancers.

Study	Country	Duration of Study	Cancer type	Detection Method	Kras Mutation N (%)	Total No. of Patients
Camps,2005 ⁴³	Spain	1999 -2002	NSCLC	Serum	20 (30)	67
Castells,1999 ⁴⁴	Spain	1996 -1997	Pancreatic cancer	Plasma	12 (28)	44
Camps,2011 ⁴⁵	Spain	NG*	NSCLC	Plasma	27 (11)	251
Chen,2010 ⁴⁶	China	2007±2008	Pancreatic cancer	Plasma	30 (33)	91
Hara,2017 ⁴⁷	Japan	2010±2013	colorectal cancer	Plasma	26 (36)	71
El Messaoudi, 2016 ⁴⁸	France	2010±2012	Colorectal Cancer	Plasma	38 (42)	91
Takai,2015 ⁴⁹	Japan	2011±2014	Pancreatic cancer	Plasma	83 (32)	259

*Not Given

CONCLUSION

Our analysis showed that the mutation of Kras found in cfDNA was a prognostic biomarker of cancer patients. Its prognostic reliability in various types of cancer was different. The principle molecular mechanism underlying this differential response is not understood and it remains to be seen whether this observation will apply to mouse models and/or human patients, but it provides an indication that downstream allele-specific targeting has potential as a therapeutic strategy. However due to the limitations in our study, there is still a need for more studies to support our conclusions.

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CONFLICTS OF INTEREST

None declared among the authors.

AUTHOR'S CONTRIBUTION

OJ, SB, SM, JM, SS conceived the idea. OJ drafted the manuscript. SM reviewed the literature. SB reviewed and approved the manuscript. All authors reviewed and approved the final manuscript.

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