

KRAS mutant colorectal tumors

Past and present

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Abbreviations: ADCC, antibody dependent cellular cytotoxicity; ASCO, America Society for Clinical Oncology; BRAF, v-Raf murine sarcoma viral oncogene homolog B1; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; GAPs, GTPase activating proteins; GEFs, guanosine nucleotide exchange factors; GRB2, growth-factor-receptor bound protein 2; GTP, guanosine triphosphate; HGFR, hepatocyte growth factor receptor; IGF-1R, insulin-like growth factor receptor type 1; IHC, immunohistochemistry; KRAS, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MAPK, mitogen-activated protein kinase; mABs, monoclonal antibodies; mCRC, metastatic colon cancer; MEK, mitogen-activated protein kinase; MET, hepatocyte growth factor receptor; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homologue deleted in chromosome 10; RTKs, receptor tyrosine kinases; SFKs, Src family kinases; SHC, SH-2 containing protein; SOS, son of sevenless homolog 1; STAT, signal transducer and activator of transcription

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The treatment of metastatic colorectal cancer (mCRC) remains one of the largest hurdles in cancer therapeutics to date. The most advanced treatment option for mCRC patients are anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (mABs) that bind to and inhibit the activity of EGFR. While the use of anti-EGFR mABs has had great impact in the treatment of mCRC, it has now been widely accepted that mCRC tumors with a mutation in the small GTPase KRAS do not respond to these therapies. KRAS mutations allow for EGFR independent activation of various oncogenic signaling cascades. In attempts to inhibit KRAS mutant tumor growth, BRAF, MEK and farnesyltransferase inhibitors have been used, however, their clinical efficacy is still accruing in the setting of CRC. Recent data suggests that various other inhibitors, including inhibitors of Src family kinases (SFK) and hepatocyte growth factor receptor (MET), may have potential preclinical and clinical success in KRAS mutant tumors. Additionally, it is becoming increasingly clear that different KRAS missense mutations may have varied biological responses to cetuximab, suggesting that cetuximab may still be a potential therapeutic option in some KRAS mutant tumors. In this review, we highlight the importance for both improved multimodality approaches for treating KRAS mutant mCRC tumors and stratification of KRAS mutations in response to different treatment regimes in order to optimize the best possible care for mCRC patients.

Introduction

Of all human cancers, metastatic colorectal cancer (mCRC) remains one of the

deadliest in the United States.¹ Upon diagnosis with CRC, 40–50% of patients demonstrate secondary metastases with an overall five-year survival period of just 11%.² With increasing need to treat mCRC patients with new therapeutic regimens, anti-epidermal growth factor receptor (EGFR) therapy, a target that is frequently overexpressed in mCRC tumors, has become a leading treatment. EGFR is a member of the HER family of growth factor receptor tyrosine kinases (RTKs). Stimulation of this receptor by various cognate ligands induces a conformational change in EGFR's extracellular domain that promotes either homo- or hetero-dimerization with other RTKs.³ Dimerization activates EGFR's intrinsic kinase activity, leading to the auto-phosphorylation of tyrosine residues on its C-terminal tail. Phospho-tyrosine residues on EGFR serve as docking sites for various adaptor and kinase proteins, many of which are known to stimulate oncogenic signaling cascades resulting in cellular survival, proliferation, migration and angiogenesis.³ To date, inappropriate EGFR activation has been linked to the development, progression and metastatic spread of various cancers.^{4,5}

Due to the high percentage of solid tumors overexpressing the EGFR, the FDA has approved five molecular targeting agents directed to block EGFR function. Of these five drugs, the anti-EGFR monoclonal antibodies (mABs) cetuximab (ICM-225, Erbitux; ImClone Systems) and panitumumab (Vectibix; Amgen) have been FDA approved for treatment of mCRC. Cetuximab is a chimeric human: murine mAB that blocks EGFR regulated signaling events by binding to EGFR's ligand binding domain preventing both ligand binding and sterically hindering dimerization with other

RTKs.⁶ Additionally, cetuximab can induce EGFR degradation and antibody dependent cellular cytotoxicity (ADCC).^{7,8} Panitumumab functions similarly, however, it is a fully humanized mAb and thus may induce less ADCC response.^{9,10}

Initial trials of chemo-refractory and chemo-naïve mCRC patients treated with anti-EGFR mAbs in addition to chemotherapy demonstrated a 10–30% response rate with a 0.9-mo increase in progression free survival time.^{11,12} Additionally, treatment with anti-EGFR mAbs in the first line setting has demonstrated increased response rates, and progression free survival times over chemotherapy alone.¹³ Various studies have also demonstrated that tumors with a lack of significant EGFR expression (quantified via immunohistochemistry, IHC) may still respond to anti-EGFR mAbs.¹⁴ Thus, predicting subsets of patients that will respond positively to anti-EGFR mAbs based on EGFR expression levels has been challenging.

The RAS Family of Small Protein GTPases

One of the most powerful predictive markers for resistance to anti-EGFR mAbs are mutations in the KRAS gene.¹⁵ KRAS is a small protein GTPase that is part of a superfamily of small GTPases that contains over 154 members, all of which have been organized into five subfamilies based on their DNA sequence and function.¹⁶ The five subfamilies are: Ras, Rho, Rab, Arf and Ran. KRAS is a member of the Ras subfamily that consists of four 21 kD proteins that differ in sequence at their c-terminus: HRAS, NRAS, KRAS4A and KRAS4B. KRAS4A and KRAS4B are different splice variants produced by alternative splicing at the c-terminus of the KRAS gene; KRAS4B is the most common splice variant and is denoted in most literature as KRAS.¹⁶ All Ras proteins are activated when bound to guanosine triphosphate (GTP), a reaction that is increased by guanine nucleotide exchange factors (GEFs) that serve to open up the GTP binding site.¹⁷ When bound to GTP, Ras proteins have increased affinity for specific downstream effector molecules, many of which are kinases that initiate various intracellular signaling

cascades. Ras proteins are subsequently deactivated through the use of their intrinsic GTPase activity, which hydrolyzes GTP.¹⁷ GTPase activating proteins (GAPs) are essential for this hydrolysis process to be complete due to their ability to stabilize the high-energy transition state of this reaction.¹⁷

KRAS functions downstream of the EGFR and serves to activate critical oncogenic signaling cascades. Upon activation of EGFR, adaptor proteins such as SH-2 containing protein (SHC) and growth-factor-receptor bound protein 2 (GRB2) recruit specific GEFs like son of sevenless homolog 1 (SOS1) to the cell membrane.^{4,16} KRAS is intrinsically targeted to the cell membrane through farnesylation and geranylgeranylation of its c-terminal tail.¹⁶ Upon association with SOS1, KRAS becomes activated and serves to further activate both phosphatidylinositol 3-kinase (PI3K) and RAF kinase, resulting in signaling through the PI3-K/AKT and mitogen-activated protein kinase (MAPK, also known as ERK) pathways.^{4,16} Additionally, KRAS-GTP activates exchange factors for the small GTPases Rac and Ral leading to the modulation of cytoskeletal dynamics and vesicular trafficking.^{16,18} Thus, KRAS is a critical mediator of EGFR induced signaling cascades (Fig. 1).

KRAS Mutations and Clinical Outcome

KRAS mutation has been documented in 35–40% of colorectal tumors, while NRAS and HRAS mutations are less common in this cancer (1–3%).^{15,16,19} The most common mutations found in KRAS are missense mutations leading to amino acid substitutions at codons 12, and 13 of exon 2, with mutation at codon 12 being most prevalent and tumorigenic in colon cancer.^{15,20} The most common amino acid substitution at both codon 12 and 13 is a glycine to an aspartate residue.^{15,21} Additionally, mutations in codons 61, 146 and 154 have been documented but are rare.¹⁵ All of these mutations promote the oncogenic potential of KRAS by (1) disabling the intrinsic GTPase activity of KRAS, and (2) preventing GAPs from associating with

KRAS.¹⁵ Thus, mutant KRAS cannot hydrolyze GTP to GDP, and therefore cannot be shut down readily leading to EGFR independent increases in the activation of both PI3K/AKT and MAPK pathways (see Fig. 1). Additionally, there has been a high prevalence of activating mutations in the BRAF gene (~15% of mCRC patients) and PIK3CA p110 PI3K subunit (~13%), along with loss of the PTEN phosphatase (~20%) in colorectal tumors.² BRAF is a serine threonine kinase that is directly activated by KRAS. Mutations in BRAF are considered mutually exclusive to those of KRAS mutations since they both constitutively activate the MAPK pathway.²

Due to the EGFR independent activation of KRAS upon its mutation, it is not surprising that anti-EGFR mAbs have provided little clinical benefit in this setting. In over 10 clinical studies, mCRC patients treated with anti-EGFR antibodies responded better if they harbored a wild type KRAS allele.¹⁵ Mutant KRAS patients treated with anti-EGFR antibody therapy had little to no response (< 10%), and one study reported detrimental effects to some patients.^{15,22} Thus, in January of 2009, the American Society for Clinical Oncology (ASCO) published a series of guidelines that strongly suggested that anti-EGFR antibody therapy be used only in the setting of wild-type KRAS.²³

Targeting KRAS Mutant Tumors: Past and Present

KRAS mutant mCRC patients have little option post chemotherapy failure. To better combat KRAS mutant tumors, researchers have tried to inhibit alternative downstream kinases. BRAF inhibitors have been in clinical trials since 2004. Initial studies with the partial RAF inhibitor sorafenib (nexavar) and more selective RAF inhibitor PLX4032 (vemurafenib) demonstrated little advantage in open trials of mCRC patients.^{24,25} Soon after, it was shown that antitumor effects were seen in a select group of mCRC patients with a V600E mutation in the BRAF gene.^{26,27} Hatzivassiliou et al. further demonstrated that the tumorigenicity of BRAF wild type, KRAS wild type, and KRAS mutant mCRC cell lines can

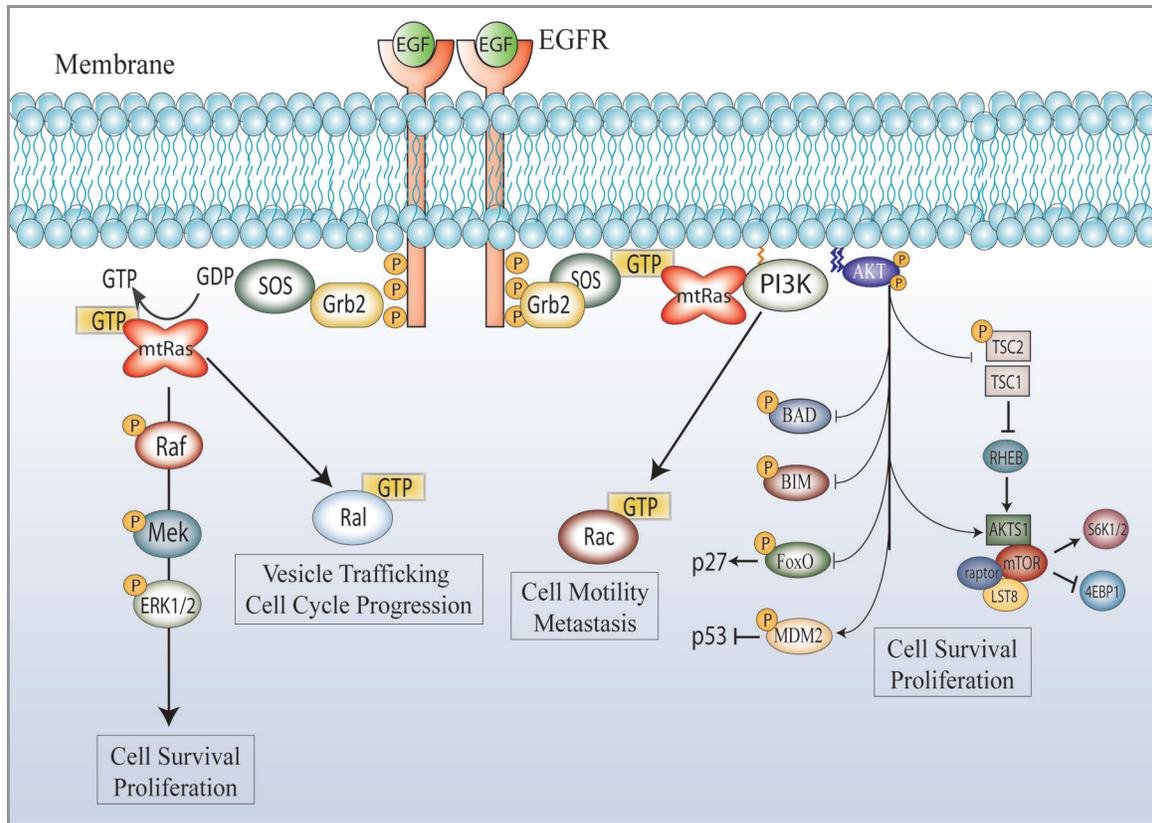


Figure 1. KRAS mutation and activation of down stream signaling cascades. In the KRAS wild-type setting EGFR activation recruits SOS to its C-terminal tail via the adaptor protein Grb2.⁴ SOS is a GEF that can activate KRAS by promoting the exchange of GDP for GTP.¹⁶ Activation of KRAS leads to a conformational change in RAF kinase, which will ultimately lead to the activation and nuclear transport of MAPK. Activated KRAS can also directly recruit PI3K to the cell surface, leading to the activation of the serine/threonine kinase AKT, resulting in the further activation of MTOR and inhibition of various pro-apoptotic signals.⁴ Additionally, KRAS can recruit various GEF proteins responsible for the activation of the small GTPases RAL and RAC.¹⁶ Through the activation of these various intracellular proteins, KRAS serves to initiate a broad oncogenic expression program that results in cellular proliferation, survival, and migration.⁴ In the KRAS mutant setting (mtRAS), KRAS is constitutively bound to GTP, and cannot hydrolyze GTP due to mutations in the GAP binding domain. In this setting, the signals emanating from KRAS are hyper-activated, and EGFR has lost its ability to control KRAS activation deeming EGFR inhibitors ineffective.

actually be enhanced by BRAF inhibition due to the activation of various oncogenic feedback loops, and thus patients should be highly selected for BRAF mutation V600E in order to receive this treatment.²⁸ Overall, BRAF inhibitors may only be beneficial for BRAF mutant mCRC patients and may explain the low response rates in past trials due to the heterogeneity of the patient population treated.

In addition to BRAF inhibition, MEK 1 and 2 inhibition has also been considered. MEK kinases are activated by BRAF. MEK kinases phosphorylate and activate MAPK. Laboratory research has shown positive outcomes in both KRAS and BRAF mutant cell lines treated with MEK inhibitors. In an in vitro and mouse xenograft model, Solit et al. demonstrated that sensitivity to MEK inhibitors was

specific for cell lines with the single BRAF mutation V600E.²⁹ Yoon et al. further showed in a KRAS mutant isogenic mCRC cell and xenograft model that the MEK inhibitors AS703026 and AZD6244 could inhibit tumor cell growth.³⁰ In another preclinical study, the very specific MEK inhibitor CI-1040 (PD 184352) demonstrated a broad range of anti-tumorigenic effects in vitro and in vivo models, especially in the setting of pancreatic cancer.^{31,32} Currently, various MEK inhibitors are being evaluated for their clinical efficacy. A phase II study by Rinehart et al. demonstrated that the MEK inhibitor CI-1040 was well tolerated by patients, however, had very little antitumor effect in the patients treated.³³ A subsequent Phase II clinical trial in mCRC patients showed that the MEK

inhibitor AZD6224 (selumetinib) had similar outcomes to treatment with the chemotherapeutic capecitabine, demonstrating possible antitumor effects of this drug, however this still needs to be further validated.³⁴ The potential for using these small molecule MEK inhibitors have also been hindered by studies modeling primary resistance to AZD6244, which lead to amplification of the mutant BRAF600E and KRASG13D genes, leading to increased signaling through the MAPK pathway.³⁵ Overall, it seems that MAPK pathway inhibition in the setting of mutant KRAS mCRC tumors remains elusive.

Other methods to inhibit KRAS activation have been to prevent its association with the plasma membrane where it becomes activated. Farnesyltransferase

inhibitors block the ability for Ras proteins to be farnesylated, a posttranslational modification necessary for plasma membrane association. Unfortunately, KRAS can alternatively become geranylgeranylated, which has proven to provide the same function as farnesylation; NRAS cannot be geranylgeranylated and thus tumors harboring NRAS mutations have proven sensitive to these inhibitors.³⁶ Laboratory studies of dual treatment farnesyl- and geranylgeranylase inhibitors in a model of KRAS mutant pancreatic cancer proved successful by promoting a greater level of apoptosis, however geranylgeranylase inhibitors were toxic in mouse models suggesting that it may be inapplicable for human treatment.³⁷ Later, it was reported that these inhibitors induced apoptosis not through inhibition of KRAS activity, but partially through inhibition of RhoB GTPase.³⁶

Currently, there have been efforts to treat mCRC KRAS mutant patients with inhibitors of various other RTKs and kinases. Both insulin like growth factor receptor type 1 (IGF-1R) and the hepatocyte-growth factor receptor (MET) have been considered as potential targets due to their overexpression in mCRC tumors.^{38,39} The use of these inhibitors in KRAS mutant tumors present similar challenges as EGFR inhibitors because both IGF-1R and MET signal through KRAS. While early clinical trials with anti-IGF-1R mAbs were unsuccessful in mCRC, mAbs directed toward c-MET in addition to panitumumab demonstrated potential in the KRAS wild-type setting with a 31% response rate (compared with 21% with panitumumab alone).⁴⁰ In vitro results have suggested, however, that both IGF-1R and MET inhibitors can overcome resistance to anti-EGFR mAB therapy.⁴¹⁻⁴³ Interestingly, an in vitro study using the BRAF mutant mCRC cell line (Colo205) demonstrated that the combinatory use of both IGF-1R and MEK inhibitors proved to be effective.⁴⁴ This combination prevented EGFR and IGF-1R crosstalk, and effectively shut down both the PI3K/AKT and MAPK signaling cascades.

Another potential target in KRAS mutant tumors are the Src family kinases (SFKs), which are overexpressed, overactive

and a marker for poor clinical outcome in mCRC.⁴⁵ Preclinical data has demonstrated that targeting SFKs with the pan SFK inhibitor dasatinib (Sprycel, Bristol-Myers Squibb) concomitantly with cetuximab in KRAS mutant cell lines and xenograft models resulted in growth inhibitory effects. Through the use of a human phospho-kinase array, researchers demonstrated that dual drug treatment lead to a decrease in components of the MAPK and β -catenin pathways and a decrease in expression of various STAT family transcription factors. In xenograft models, dual drug treatment decreased proliferation and enhanced apoptosis, suggesting that dasatinib could sensitize KRAS mutant mCRC tumors to cetuximab.⁴⁶ Overall, these studies demonstrate the importance for dual kinase inhibition in the setting of mCRC, and also indicate that EGFR may still be a drugable target in the KRAS mutant setting.

Finally, it is becoming increasingly clear that cetuximab may actually increase both overall and progression-free survival in patients that have a mutation in KRAS at codon 13.⁴⁷ In a study by De Roock et al., a pooled data set of 579 mCRC patients across various clinical trials treated with cetuximab plus/minus chemotherapy demonstrated that overall and progression-free survival was significantly longer in patients with G13D KRAS mutant tumors. Patients with G13D KRAS mutant tumors and treated with cetuximab/chemotherapy regimes had overall survival and progression free survival of average 7.6 and 4.0 mo vs. 5.7 and 1.9 mo in other KRAS mutant tumor subtypes. This year at ASCO, Tejpar et al. further supported this finding by presenting retrospective analyses of two large phase III multicenter trials (CRYSTAL and OPUS) representing 83 patients with G13D KRAS mutant mCRC tumors who had longer overall survival and progression free survival on average post cetuximab treatment than other KRAS mutant subtypes.²¹ Overall, these data suggest that anti-EGFR mAbs should still be considered as treatment options for patients with a G13D KRAS mutation, and that stratification of different KRAS mutant subtypes should now be documented.

Future Directions

Key questions remain unanswered in the field of mCRC therapy and the role of KRAS in this setting. First and foremost, we must identify the key factors that influence the oncogenicity of KRAS mutant tumors. The apparent lack of response to MAPK pathway inhibitors in mCRC suggests that KRAS signals through multiple oncogenic pathways to influence tumorigenicity, and that MAPK pathway inhibition may not be sufficient to inhibit tumor growth. Thus, it is essential to focus on multiple pathway inhibition, and to continue discovery of other unrelated activated pathways in this setting. Second, we must continue to stratify the important predictive factors for response to different targeted therapies. Identification of mutations in the KRAS and BRAF genes as negative predictive markers for response to anti-EGFR mAbs has made a great impact in the field, and has prevented patients from receiving useless therapy. However, recent data suggests that EGFR may still be a target in this setting. It is becoming more apparent that different missense mutations (codon 12 vs. 13) in the KRAS gene are not equal in their transforming potential. Thus, further preclinical and clinical trials are necessary in order to devise the best treatment options for specific mutant KRAS tumor subtypes. At present, mCRC patients with a mutant KRAS gene are in dire need for better treatment options, and thus both clinicians and research scientists alike must work together to make this a reality.

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