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CCR New Strategies

Clinical Cancer Research

New Strategies for Treatment of KRAS Mutant Metastatic Colorectal Cancer

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Abstract

The introduction of new cytotoxic agents and new targeted therapies has significantly broadened the therapeutic options for and the outcomes of patients with metastatic colorectal cancer (CRC). The introduction of the anti-epidermal growth factor receptor (EGFR) antibodies, cetuximab and panitumumab, has clearly contributed to this development. The concept of KRAS as a marker for resistance to anti-EGFR antibodies has been validated. However, new challenges have emerged: the complete understanding of the crucial and central role of KRAS in processes of tumor growth and the development of new treatment strategies for KRAS mutant tumors. KRAS seems to be so crucial that a further classification and description in KRAS wild-type and mutant may be warranted. Testing for *KRAS* mutations marks, therefore, a paradigm shift in the management of metastatic CRC. This testing also highlights the unmet need for new treatment options in KRAS mutant metastatic CRC. In this review we will focus on possible new treatment options for these patients. *Clin Cancer Res; 16(11); 2921–6. ©2010 AACR.*

Background

Metastatic colorectal cancer (mCRC) continues to have a dismal prognosis unless the metastases are resectable, despite clear progress in chemotherapeutic options. At present, a combination of a fluoropyrimidine [5-fluorouracil (5-FU)] or capecitabine with either oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) is the backbone of the treatment of patients with mCRC (1). Recently, monoclonal antibodies (MoAb) that target vascular endothelial growth factor (VEGF; bevacizumab) and epidermal growth factor receptor (EGFR; cetuximab and panitumumab) have been approved for the treatment of mCRC. Adding bevacizumab, a humanized MoAb targeting VEGF, to regimens containing 5-FU, irinotecan, or oxaliplatin improves outcome in the first-line as well as in the second-line setting of mCRC and is, therefore, now considered a standard first-line treatment option for unselected mCRC (2, 3).

The EGFR signaling pathway has been the focus of new drug development of EGFR inhibitors for CRC. Cetuximab, a chimeric immunoglobulin G1 (IgG1) MoAb against the EGFR, was the first EGFR inhibitor to be approved for clinical use for mCRC. It binds the EGFR with high affinity and competitively inhibits ligand binding, which leads to inhibition of phosphorylation and subsequent activation of downstream signaling pathways. Both single agent cetuximab as well as the combination with irinotecan have shown activity in patients with mCRC (4–8). Similar results have been obtained with panitumumab, a fully human antibody directed against EGFR (9–11).

When the EGF or other ligands occupy the EGFR, it activates a signaling cascade via several pathways, including the RAS-RAF-mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)-AKT pathways (Fig. 1; ref. 12), which control cell proliferation, differentiation, and survival. RAS is one of the most important molecules in the EGFR downstream signaling pathway (13). Three human RAS genes have been identified: *HRAS*, *KRAS*, and *NRAS*. They are small GTP-GDP–binding proteins that act as functional switches by coupling growth factor receptors to intracellular signaling pathways. RAS can activate the kinase RAF, the mitogen-activated extracellular signal-regulated kinases (ERK)1 and ERK2, PI3K, and many other proteins to promote cell proliferation (13). Because activation of the EGFR leads to activation of the intracellular effector KRAS, it was hypothesized that mutations in the *KRAS* coding gene could lead to a constitutively activated KRAS protein that is independent from upstream signals, which subsequently could affect clinical response to EGFR inhibitors. Mutations in the *KRAS* proto-oncogene occur in approximately 35 to 40% of CRC (14), are single nucleotide point mutations mostly in codons 12 and 13 of exon 2 (15), and are early events during the development of CRC carcinogenesis. The incidence of KRAS mutations is identical throughout all stages (16), and a very high concordance has been reported between paired primary cancers and metastatic samples (17).

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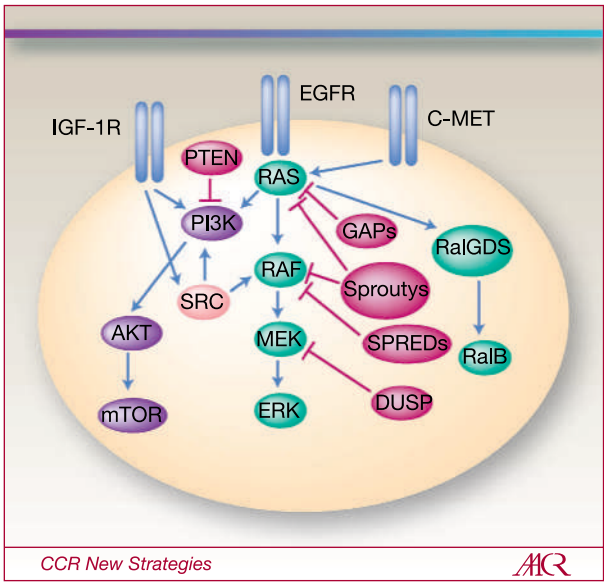

Fig. 1. Overview of the most important pathways that can be targeted in KRAS mutant CRC.

There is no complete agreement on the prognostic role of KRAS mutations. Recently several studies have shown that KRAS mutations are not prognostic in stage II or III colon cancer (18–21). In mCRC, if prognostic at all, patients with a KRAS mutant tumor have only a slightly worse outcome, in contrast to BRAF mutations that are clearly prognostic. Retrospective studies showed that *KRAS* mutations in codon 12 or 13 are associated with lack of response to cetuximab in patients with chemorefractory mCRC (22–25). Moreover, there is evidence in multiple randomized trials of improved response rate, progression free survival and/or overall survival in response to anti-EGFR MoAb therapy only in patients with no mutations in codon 12 or 13 versus mutated KRAS tumors. Since then, *KRAS* mutations have emerged as a major predictor of resistance to EGFR inhibitors in first-line as well as in subsequent lines of treatment (8, 26–32). Moreover, two clinical trials even show a possible detrimental effect of treatment with an EGFR inhibitor in combination with oxaliplatin in patients with mutated KRAS tumors (11, 32). The reason for this effect is not yet clear. The American Society of Clinical Oncology (ASCO) recently published their provisional clinical opinion that patients with mCRC, having a KRAS mutation in codon 12 or 13, should not receive anti-EGFR antibody treatment (33). Moreover, methodological aspects of KRAS testing and the type of assay are important, to have a good balance between accuracy and practicality (33, 34). The role of rare mutations in KRAS (codon 61 and 146) and NRAS in resistance to EGFR inhibitors still needs to be elucidated but will be difficult because of their low frequency.

Not all patients with a KRAS wild-type genotype respond to EGFR-targeted agents. Recently, activating mutations of *BRAF*, which encode for a protein acting downstream of KRAS, were also shown to be responsible for resistance to EGFR inhibitors in chemorefractory CRC (24, 35, 36). A recent publication confirms, as for KRAS, a high concordance of BRAF mutations in primary CRC and related metastatic sites (37).

Development of new therapeutic strategies for KRAS mutant tumors, are therefore highly needed. Because the KRAS pathway is central to many nodes of receptor tyrosine kinase (RTK) signaling, the same hurdles are to be expected in inhibition of other RTKs. In this overview, we focus on other molecular targeted therapies that theoretically could be interesting for treating CRC patients with KRAS mutated tumors.

On the Horizon

The oncogene *KRAS* is the most commonly mutated gene in various human cancers. Being constitutively activated, it can bypass the EGFR-driven signaling cascade and impair the clinical efficacy of EGFR inhibitors. In theory there are several possible ways trying to overcome this resistance, which we will discuss in detail, together with the possible limitations of these approaches. Figure 1 shows an overview of the most important pathways that can be targeted.

Targeting Molecules Downstream of RAS

RAF Inhibitors

RAF kinases are serine-threonine protein kinases that function as downstream effector molecules of RAS and initiate a mitogenic kinase cascade leading to cell proliferation (38). The RAF kinase family is composed of three members: ARAF, BRAF, and CRAF. *BRAF* mutations, occurring in 5 to 10% of CRC, and *KRAS* mutations are mutually exclusive. Because RAF is an important effector downstream of RAS in the ERK signaling pathway, it could be a potential target for treating KRAS mutated tumors. One of the first inhibitors of RAF activity is sorafenib, which is a multikinase inhibitor with activity against CRAF, wild-type BRAF, the V600E mutant form of BRAF, vascular endothelial growth factor receptor (VEGFR) and platelet derived growth factor receptor (PDGFR; ref. 39). It has received approval for the treatment of advanced renal cell carcinoma and hepatocellular carcinoma. Sorafenib, however, is a relatively weak RAF inhibitor, which is a possible explanation for disappointing early clinical trials with this inhibitor in melanoma (40). Sorafenib is rather an inhibitor of angiogenesis by inhibiting VEGFR-1, -2, -3, and PDGFR. More promising are the potent and selective RAF inhibitors such as PLX4032, which showed high activity in a phase I study in melanoma harboring an activating mutation in BRAF (41).

The treatment of KRAS-mutated CRC with a selective BRAF and/or CRAF inhibitor could be an interesting approach.Phase II clinical trials are currently ongoing with the combination of sorafenib with either FOLFOX,

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FOLFIRI, or cetuximab (42). The more specific RAF inhibitors are currently under early clinical development (41, 43).

MEK Inhibitors

Activated RAF causes the phosphorylation and activation of mitogen-activated protein kinase (MAPK) extracellular signal regulated kinases 1 and 2 (MEK1 and MEK2), which in turn phosphorylate and activate ERK1 and ERK2. ERK then migrates to the nucleus and activates several nuclear transcription factors, which is important in stimulating cellular proliferation, differentiation, and survival. Selective inhibitors of the MEK kinases seem an attractive target for tumors that preferentially signal through the RAS-RAF-MEK-ERK pathway. It has been shown that melanoma cell lines with mutant BRAF are very sensitive to MEK inhibition (44). Surprisingly, Solit et al. also found that cell lines harboring the oncogene RAS showed much lower and more variable sensitivity to MEK inhibition. A possible explanation for this finding could be that signaling from RAS bifurcates to several downstream targets in addition to BRAF-MEK. Thus inhibition of MEK may not be sufficient, at least in some situations, which is reflected in clinical studies in which MEK inhibitors show only limited antitumor activity (45). Recently it has also been shown that a compensatory or activating feedback loop between RAF-MEK-ERK and PI3K pathways counteracts the effect of MEK inhibition (46). Moreover, it was found that dual inhibition with MEK and PI3K inhibitors resulted in marked tumor cell growth inhibition. Preclinical experiments in CRC cell lines showed that the sensitivity to MEK inhibitors varied extensively and that either activating mutations in *PIK3CA* or loss-of-function mutations in PTEN resulted in insensitivity to the MEK inhibitor (47). The authors conclude that PI3K pathway activation is a major resistance mechanism that impairs efficacy of MEK inhibitors in *KRAS* mutated cancers. Together with the previous study, it provides a strong rationale for the combination of PI3K and MEK inhibitors.

In conclusion, although MEK inhibition is theoretically an interesting approach to target KRAS activated tumors, it is very likely that MEK inhibitors may only be efficient in a subgroup of KRAS mutant CRC. As we will discuss later in this manuscript, combination with other targeted agents is probably a more efficient approach. Phase I and II clinical trials are currently ongoing with more than five different MEK inhibitors. A recent article reviews the current status of MEK inhibitors in clinical development (48).

Targeting the PI3K/AKT/Mammalian Target of Rapamycin Pathway

PI3K can be activated both by RTKs [(such as EGFR, c-MET, insulin-like growth factor (IGF)] as by RAS itself (49). AKT is the chief mediator of downstream signaling through various targets such as mammalian target of rapamycin (mTOR). Because the oncogene KRAS can also activate this pathway and this has been suggested as a possible resistance mechanism for MEK inhibitors, it makes sense to study inhibitors of the PI3K-AKT-mTOR pathway for the treatment of KRAS-mutant CRC. As described above preclinical data support the use of the combination of MEK and PI3K inhibitors for this purpose. Proof of concept was provided in a preclinical study with NVP-BEZ235, a dual pan-PI3K and mTOR inhibitor, which inhibited tumor growth in mouse lung cancers driven by mutant KRAS, when combined with an MEK inhibitor (50).

Many inhibitors of PI3K, AKT, and mTOR are currently in clinical development (phase I and II trials). A possibly interesting approach for targeting KRAS mutant CRC, is the inhibition of the more downstream target of the PI3K pathway mTOR in combination with a MEK inhibitor, which is currently the basis of a phase I trial supported by GlaxoSmithKline (42).

Rationale for Combining Targeted Agents

It has become clear that activation of RAS results in activation of very complex branching pathways. Theoretically, blocking one downstream target of RAS will not be sufficient to result in inhibition of tumor growth. A nice example is the study by Wee and colleagues, in which it is shown that PI3K pathway activation renders *KRAS* mutant less sensitive to MEK inhibitors (47). Moreover, they found that the extent of resistance conferred by *PIK3CA* and *PTEN* mutations was different, with loss of *PTEN* function leading to complete resistance. These findings support the fact that mutational activation of *PIK3CA* is not functionally equivalent to loss of *PTEN*. Therefore, they conclude that is important to test if pan-PI3K inhibitors will synergize with MEK inhibitors in cancers with coexisting *PTEN* and *KRAS* mutations.

Another important issue to take into account is the presence of negative feedback loops. Mirzoeva and colleagues studied the molecular features of breast cancer cell lines that are sensitive to pharmacological inhibition of the MEK-ERK signaling pathway (46). They discovered a negative feedback loop, activating AKT in response to MEK inhibition in an EGFR-dependent fashion, thus amplifying EGF signals. Dual inhibition of MEK and PI3K led to synergistic inhibition of growth of these cell lines.

Negative feedback loops, such as the induction of phosphate-removing enzymes that target ERK, can attenuate the steady-state phosphorylation of ERK, which means that other targets might also be more important in certain circumstances. Until recently, research has focused more on tyrosine kinase. However, recent research has also focused on protein tyrosine phosphatases, which has led to a better understanding of the tightly controlled balance of reversible protein phosphorylation (51). The dual-specificity phosphatases (DUSP) are able to dephosphorylate both tyrosine and serine-threonine residues within one substrate. One of the best-characterized DUSP subgroups

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are the MAP phosphatases, which can dephosphorylate MAPK and thereby inactivate ERK. The expression of this feedback loop could reflect the activity and importance of the MAPK pathway. For example, we recently showed that DUSP4 (an MPK) expression levels correlate with overall survival in CRC patients treated with cetuximab ± irinotecan. In KRAS wild-type patients, low DUSP4 levels were favorable, whereas in KRAS mutant, high DUSP4 levels are found (52). This crosstalk between pathways and the presence of multiple nodeswith feedback loops highlights the importance of targeting multiple downstream kinases rather than just one. Perturbing these feedback loops can have dramatic effects on drug responses. For example, by targeting MEK in tumors, in which a feedback loop is activated, the net effect can be an increase in EGFR signaling, which could ultimately enhance tumor growth (53). On the other hand, in cancers bearing mutant RAS, blocking the PI3K pathway can upregulate signaling of the RAF-MAPK pathway because the two pathways have cross-inhibitory effects (54). Combined inhibition may be a solution to this problem. This approach was recently confirmed in preclinical studies where blocking this feedback mechanism through targeting MEK and EGFR resulted in synergistic effects in some gastric cancer cell lines *in vitro* and *in vivo* (55).

However, we have to take into account that combining multiple tyrosine kinase inhibitors (TKI) can also have a detrimental effect, as was recently shown by combining EGFR and VEGFR inhibitors (56, 57); the reason for this effect is not known.

In conclusion, a dual-targeted or multitargeted strategy may be more efficient to eliminate cancer cells and to fight drug resistance.

Targeting Other Receptor Tyrosine Kinases

Another possible approach to target KRAS mutant tumors is by blocking other important RTKs that contribute to enhanced cell survival and proliferation. In KRAS mutant patients, part of the prosurvival pathway could still be by activation of more upstream RTKs other than the EGFR. IGF-1 and MET receptor are two promising targets, and inhibitors are in early clinical development in mCRC.

The type 1 IGF receptor (IGF-1R) signaling pathway is an important pathway in different types of cancers including colon cancer (58). Recent evidence suggests a role for IGF-1R signaling in the acquired resistance to EGFR inhibitors in glioblastoma cells (59). There is a lot of evidence for cross-talk between IGF-1R and EGFR (60). The presence of this cross-talk may be of importance in anticancer therapy. Preclinical data showed that combination treatment of IGF-1R and EGFR kinase inhibitors resulted in synergy of growth inhibition in CRC cell lines (60). It is likely that blocking one of both receptors will lead to reshuffling of the downstream signaling pathways and affect the other receptor. Because the IGF-1R is coupled to several intracellular second messenger pathways, including the PI3K signaling pathway, an antitumor effect in KRAS mutant CRC is theoretically possible, although unclear today. Multiple IGF-1R inhibitors (both MoAb and TKIs) are currently under clinical evaluation.

The hepatocyte-growth factor (HGF)–mesenchymal-epithelial transition factor (MET) molecular pathway is well known as an important pathway in cancer development. MET-related signal transduction is thought to be involved in the development of resistance to EGFR targeting agents (61). The combinatorial inhibition of HGF-MET and EGFR is therefore an interesting approach to assess in clinical trials. In a recent review, the issue of targeting MET as a strategy to overcome cross-talk–related resistance to EGFR inhibitors was summarized very well (62).

But, because KRAS lies central to many nodes of RTK signaling, the same hurdles are to be expected in inhibition of other RTKs, such as the IGF-1R and MET pathway. Therefore in KRAS mutant patients, a combination of MET or IGF-1R inhibitors, together with inhibitors of targets more downstream than KRAS, is probably a more interesting approach for treating these patients.

Other Approaches

Bevacizumab is active in mCRC in combination with a cytotoxic backbone, regardless of the KRAS mutation status. A relatively small retrospective analysis has indeed shown that the benefit is similar in KRAS wild-type and mutant tumors (3). Bevacizumab is therefore a therapeutic option against KRAS mutant tumors.

Lenalidomide is an analog of thalidomide. However, whereas lenalidomide is able to enhance antibody-dependent cellular cytotoxicity (ADCC) *in vitro*, the effect of thalidomide is minimal. Furthermore, lenalidomide retains good anti-angiogenic activity *in vitro*. Lenalidomide enhances natural killer (NK) cell and monocyte-mediated ADCC of rituximab against a variety of hematological cell lines *in vitro*, including NHL and B-CLL (63). Lenalidomide also enhances NK-cell–mediated lysis of cetuximab and trastuzumab-coated colorectal and breast cancer cells, respectively (63). The ability of lenalidomide to enhance cetuximab-mediated ADCC of CRC cells is not affected by the KRAS mutational status. Thus, KRAS wild-type and KRAS mutant CRC cells are equally sensitive to enhancement of ADCC by lenalidomide (63). This result is as expected because NK cells recognize the surface-bound antibody and are able to kill tumor cells independently of EGFR pathway activation. Because panitumumab, an IgG2a EGFR MoAb, does not effectively interact with FCγ receptors on the NK cell surface, it is unable to initiate ADCC and as expected lenalidomide has no effect because its activity is reliant on the augmentation of NK cell signaling downstream of FCγR. An early clinical study exploring this effect of lenalidomide in KRAS mutant tumors has been initiated.

Finally, farnesyltransferase inhibitors (FTI) were developed to specifically inhibit the activity of oncogenic RAS. However, subsequent studies showed that FTI-mediated

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inhibition of tumor growth was not tightly linked to *RAS* mutation status (64). Clinical trials with FTIs were disappointing (65). It is hoped that FTIs, whereas not RAS specific, still have potential for cancer therapy.

Conclusion

KRAS plays a central role in the process of CRC tumor growth. There is an unmet need of new therapeutic strategies for KRAS mutant tumors as they don't respond to EGFR-targeted agents. Blocking one downstream target of RAS is in theory an interesting approach, but will probably not be sufficient to result in inhibition of tumor growth as it signals through very complex branching pathways. A dual-targeted or multitargeted strategy may therefore be more efficient, but this still needs to be confirmed in clinical trials, which are currently ongoing. It is important to include translational research in the ongoing trials for the evaluation of potential biomarkers, which subsequently can be validated prospectively.

Disclosure of Potential Conflicts of Interest

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