REVIEW

From basic researches to new achievements in therapeutic strategies of KRAS-driven cancers

Mahsa Sali ani 1, Razieh J al al 1,2, Mohammad Reza Ah madian 3

1Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Mashhad 9177948974, Iran; 2Department of Research Cell and Molecular Biology, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad 9177948974, Iran; 3Institute of Biochemistry and Molecular Biology II, Medical Faculty, Heinrich-Heine University, Düsseldorf 40225, Germany

ABSTRACT

Among the numerous oncogenes involved in human cancers, KRAS represents the most studied and best characterized cancer-related genes. Several therapeutic strategies targeting oncogenic KRAS (KRAS onc) signaling pathways have been suggested, including the inhibition of synthetic lethal interactions, direct inhibition of KRAS onc itself, blockade of downstream KRAS onc effectors, prevention of post-translational KRAS onc modifications, inhibition of the induced stem cell-like program, targeting of metabolic peculiarities, stimulation of the immune system, inhibition of inflammation, blockade of upstream signaling pathways, targeted RNA replacement, and oncogene-induced senescence. Despite intensive and continuous efforts, KRAS onc remains an elusive target for cancer therapy. To highlight the progress to date, this review covers a collection of studies on therapeutic strategies for KRAS published from 1995 to date. An overview of the path of progress from earlier to more recent insights highlight novel opportunities for clinical development towards KRAS onc-signaling targeted therapeutics.

KEYWORDS

Direct inhibition; downstream effectors; oncogenic KRAS; drug target sites; small GTPases; signal transduction; targeting synthetic, lethal interactions; therapeutic strategies

Introduction

KRAS is a small GDP/GTP-binding protein that transduce extracellular signals into intracellular responses. It cycles between an inactive, GDP-bound ("off") state and an active, GTP-bound ("on") state. This off/on cycle is tightly regulated by RAS-specific guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) 1. In its active state, KRAS binds and activates various effector proteins function and thus regulate downstream signaling pathways (Figure 1). The conserved GDP/GTP-binding (G) domain of KRAS contains two flexible regions, the switch regions 1 and 2, which provide a functional platform for the interaction with regulators and effectors 2-4. The C-terminus of KRAS, which is highly variable among the RAS paralogs, is the site for post-translational modifications and responsible for KRAS anchorage to the plasma membrane 5-7.

Upstream signaling pathways of KRAS are activated by binding of ligands to their transmembrane receptors, mostly receptor tyrosine kinases, and recruitment of docking proteins, such as GRB2, in complex with RAS-specific GEFs, which facilitates KRAS activation (Figure 1) 8-10. GTP-bound KRAS further transduces the signal to its downstream effectors and thus activates multiple signaling pathways 11-15. Thereby, KRAS controls various cellular processes, including survival, growth, proliferation, differentiation, and apoptosis 16-18.

With the discovery of the mutational activation of RAS genes in human cancers dating back to the 1960s, extensive studies have been conducted to understand the localization, regulation and signaling of RAS proteins with the ultimate goal of developing anti-RAS drugs for cancer treatment 3. Somatic mutations, most frequently identified KRAS4B (oncogenic KRAS or KRAS onc) (COSMIC), contribute to robust gain-of-function effects and to various types of cancers as well as leukemia and lymphoma tumors 19-22. Due to reduced GTP hydrolysis and resistance to GAPs 19,20, KRAS onc persist in a constitutive active state and thus, strongly contribute to neoplastic signal transduction 23.

Despite intensive efforts on the understanding of the
mechanisms of intracellular trafficking, regulation and signaling activity of RAS proteins, specific inhibition of oncogenic RAS has not been clinically established to date. Among the RAS protein family, KRAS mutations are the most common oncogenic driver in many human cancers. Additionally, KRAS onc is a strong predictive biomarker of resistance to anti-EGFR (Epidermal Growth Factor Receptor) treatment. Therefore, the prevalence of KRAS mutations in a number of human cancers and its inherent resistance to anti-EGFR targeting underscores the clinical relevance of targeting KRAS onc in cancer treatment.

Extensive research on different cell lines harboring the KRAS mutation have been conducted, including a pancreatic cancer cell line (PANC-1), human colorectal cancer cell lines (DLD-1, HCT-116, and Colo-320 cells), non-small cell lung cancer (H441 cells), human bronchial epithelial cells (HBEC3KT cells), human alveolar basal epithelial cells (A-549 cells), human oral squamous cell carcinoma (H157 cells), human breast adenocarcinoma cells (MCF-7 and SKBR3-LR cells), murine embryonic fibroblasts (MEFs), and acute myeloid leukemia cells (NOMO-1). According to studies on targeting the KRAS oncogene, therapeutic strategies can be divided into two main categories: 1) small molecule inhibitors, which are synthetically lethal to mutant KRAS or designed to prevent the post-translational processing of KRAS onc, upstream pathways, KRAS onc/GEF interactions and downstream KRAS' effectors; and 2) anti-KRAS genetic therapies, which interfere with the expression of KRAS or other components of KRAS onc-associated signaling pathways.

The complexity of KRAS signaling pathways, in which KRAS protein interacts with many different upstream mediators, downstream effectors, and transcription factors in a nonlinear fashion, has a critical role in the lack of effective targeting strategies.
a better understanding of KRAS interactions with other proteins and transcription factors may provide new opportunities for effective treatment (Figure 1).

In this review, we provide a snapshot view of the rich history of KRAS research by chronologically discussing representative key retrospective discoveries regarding the various therapeutic options for cancers associated with KRAS mutations. In addition to basic original anti-KRAS onc therapeutic mechanisms, novel approaches, including inhibition of the embryonic stem cell-like program\(^{18}\), targeting of upstream tyrosine kinases\(^{10}\), stabilization of KRAS\(^{onc}\) G-quadruplex structures\(^{35}\), inhibition of inflammation\(^{36}\), and targeting of metabolic peculiarities\(^{37}\), for suppression of aberrant KRAS activation in cancers are also explained (Figure 2).

In addition to KRAS mutations, amplification of wild-type KRAS gene or EGFR mutation leads to the over-expression or over-activation of KRAS, respectively. Some studies have shown that both over-expressed KRAS and KRAS\(^{onc}\) can be associated with aggressive and metastatic cancer phenotype\(^{38,39}\). Regarding these similarities, some of the targeting strategies discussed in this review may be applied for both KRAS and KRAS\(^{onc}\), e.g., inhibition of downstream signaling pathways or inhibition of plasma membrane localization. In contrast, structural differences between KRAS\(^{onc}\) and KRAS provide distinct therapeutic opportunities\(^{40}\). Some studies, which are referred to in this review, focus on total RAS proteins. Considering that the KRAS mutation represents approximately 90% of identified RAS mutations\(^{33}\), the results of studies on total RAS proteins could certainly be applied to KRAS protein.

### Inhibition of KRAS localization

KRAS localization in the plasma membrane is a critical step

---

**Figure 2** Different therapeutic targets for KRAS driven cancers. The most important of these therapeutic strategies discussed in this article are shown by numbers: (1) Inhibition of transcription by G4 elements. (2) Inhibition of translation through complementary microRNAs. (3) Targeting enzymes posttranslationally modifying KRAS. (4) Targeting KRAS membrane trafficking. (5) Interference with upstream signaling by targeting of receptor tyrosine kinases. (6) Targeting GEFs and RAS activation. (7) Targeting KRAS effectors and downstream signaling pathways. (8) Suppression of synthetic lethal interactions. (9) Targeting inflammatory signaling pathways. (10) Targeting cell cycle progression. (11) Reregulation of metabolic alternations. (12) Reprogramming of stem cell properties. (13) Upregulation of miRs with anti-KRAS activity. Black arrows with blocked red circles are referred to inhibited targets as potential therapeutic approaches.
for its activation and signaling. Thus, inhibition of KRAS localization provides new insights for cancer treatment. There are three main approaches to prevent KRAS post-translational modifications: 1) inhibition of KRAS post-translational modifications, 2) displacement of KRAS from the membrane, and 3) impairment of proper intracellular trafficking of KRAS protein, it must undergo a series of post-translational modifications, which facilitate its association with the cell membrane.

Initially, the enzyme farnesyl transferase (FTase) catalyzes the addition of a farnesyl isoprenoid moiety to the thiol group of the terminal cysteine in the CAAX motif of KRAS protein. CAAX stands for C, a cysteine, A for aliphatic amino acids and X for any amino acid. Next, protease RAS-converting enzyme-1 (RCE-1) cleaves the terminal AAX amino acids, and then the carboxyl group of the cysteine is methylated by isoprenyl-cysteine carboxymethyl transferase-1 (ICMT-1). Multistep post-translational modifications of KRAS protein provide several possible drug targets, including FTase, RCE-1, and ICMT-1. Attempts have been made to target KRAS post-translational modifications to inhibit its membrane localization and thus its activation and downstream signaling for the treatment of cancers. Prevention of KRAS processing to form a stable interaction with the cell membrane is not the only mechanism to reduce the population of KRAS at the membrane. Displacement of KRAS from the membrane and the impairment of proper trafficking are the two other strategies. For instance, perturbation of the subcellular distribution of phosphatidylinositol-3 kinase, resulting in its displacement from the membrane. Another strategy triggering the mislocalization of KRAS is phosphorylation of S181 in the C-terminal hypervariable region (HVR) of KRAS, thereby activating the farnesyl-electrostatic switch.

Targeting post-translational modifications of KRAS to inhibit its plasma membrane localization appeared to be promising in preclinical studies; however, alternative post-translational modifications of KRAS and disruption of the prenylation of proteins other than KRAS have led to disappointing results. In spite of the earlier unsuccessful results, continuation of the studies on the disruption of KRAS plasma membrane localization has led to the development of novel treatment outcomes. For example, RAS binding proteins, such as phosphodiesterase delta subunit (PDEδ), have attracted considerable attention as a new target. Prenylation of KRAS increases its hydrophobicity and, thus, reduces its solubility. PDEδ facilitates the distribution of RAS family proteins by covering hydrophobic group. Therefore, inhibition of the RAS-PDEδ interaction prevents oncogenic RAS activation and signaling and results in an anti-cancer effect on RAS-transformed cells. In recent studies, blockade of the prenyl-binding pocket of PDEδ demonstrated promising results. In order to have a view on the progress has been made for disruption of KRAS plasma membrane localization, studies examining the blockade of KRAS processing, mislocalization, and trafficking published from 1993 to 2016 are summarized chronologically (Table 1).

## Direct inhibition of KRAS

In response to extracellular stimuli that activate cell surface receptors, RAS protein members mediate the transduction of extracellular signals to intracellular responses. Small GTPases of the RAS family function as molecular switches that cycle between active, GDP-bound and inactive, GTP-bound states. Activation of upstream signaling pathways results in the recruitment of GEFs, such as SOS1 and SOS2, which facilitate KRAS activation by catalyzing the release of GDP from KRAS. Activated KRAS controls different cellular processes that are also involved in the transformation of normal cells to the malignant phenotype.

The intrinsic GTPase activity of wild-type KRAS is enhanced by GAPs; however, oncogenic KRAS mutations lead to the impairment of GTP hydrolysis and cause GAP insensitivity and thus constitutive activation of KRAS. Indeed, inhibition of the constitutively active KRAS is a conceptually ideal strategy for cancer therapy. Two general mechanisms have been suggested for direct inhibition of RAS proteins, including decreasing the proportion of KRAS GTP in its GTP state and disrupting the KRAS-effector interactions. To decrease KRAS-GTP levels, several approaches have been used, such as the inactivation of KRAS with small molecules or GTP analogs that facilitate GTP hydrolysis activity, interference with the nucleotide exchange process through disruption of the SOS-KRAS interaction, subversion of the native nucleotide preference of the KRAS to favor GDP over GTP, irreversible inhibition of the KRAS with its covalent modification, inactivation of KRAS in the GTP state, inhibition of intrinsic nucleotide exchange, and inhibition of nucleotide binding.

Activation of downstream effectors, such as RAF kinases, is accomplished through direct interaction of KRAS with its effectors. Likewise, other approaches in treatment of KRAS-driven cancers, first generation of RAF kinase
inhibitors had limited clinical benefit where the inhibitors found to paradoxically activate ERK pathway through the induction of RAF dimerization in RAS-mutant cancers.75 Discovery programs in the development of new RAF

### Table 1  Inhibition of RAS plasma membrane localization

| Strategy                          | Target                          | Inhibitor                  | Result                                                                 | RAS type        | Cells/tissues               | Reference |
|-----------------------------------|---------------------------------|----------------------------|------------------------------------------------------------------------|-----------------|-----------------------------|-----------|
| Inhibition of post-translational modification | Ftase | FTI-277 | Inhibition of oncogenic HRAS and KRAS processing and PM localization with blocking constitutive activation of MAPK | KRAS and HRAS  | NIH3T3 fibroblasts          | 50        |
|                                   | Prenylated protein methyltransferase (PPMase) | S-trans,trans-farnesylthiosalicylic acid | Inhibition of cell growth HRAS | HRAS | Rat1 fibroblasts            | 51        |
| Ftase                            | B956                            |                            | Inhibition of human tumor xenograft growth KRAS                        | KRAS            | Colon carcinoma             | 52        |
| Ftase                            | Lonafarnib (SCH-66336)          |                            | Inhibition of soft agar and human tumor xenograft growth HRAS and KRAS | HRAS and KRAS  | NIH3T3 and lung carcinoma  | 53        |
| Ftase                            | Lonafarnib (SCH-66336)          |                            | Inhibition of colony formation of tumor cells KRAS                     | KRAS            | Colon and pancreatic cancer | 54        |
| Ftase and GGTase                 | FTI-277 and GGTI-297            |                            | Inhibition of tumor growth KRAS                                        | KRAS            | NIH3T3 and lung carcinoma  | 55        |
| Ftase                            | Lonafarnib (SCH-66336)          |                            | Cell cycle arrest in G2 to M phase (KRAS mutated cells) and in G1 phase (HRAS mutated cells) | KRAS and HRAS | Lung, colon, pancreas, and NIH3T3 | 56        |
| Ftase                            | BMS-214662                      |                            | Inhibition of growth attributed to the induction of apoptosis and curative response in human tumor xenografts HRAS | HRAS            | Colon carcinoma             | 57        |
| Ftase                            | L-744, 832                      |                            | Promotion of apoptosis and cell cycle arrest lead to inhibition of anchorage-dependent growth HRAS and NRAS | HRAS and NRAS  | Pancreatic cancer           | 58        |
| Ftase                            | FTI-2153                        |                            | Accumulation of cells in prometaphase by blocking bipolar spindle formation and chromosome alignment HRAS | HRAS            | Lung cancer                 | 59        |
| RCE-1                            | Creadenovirus excision of RCE-1 |                            | Reduction of cell growth and RAS-induced transformation KRAS           | KRAS            | Primary mouse embryonic fibroblasts and skin carcinoma | 45        |
| ICMT-1                            | Methotrexate                    |                            | Decrease in RAS methylation, mislocalization of RAS, and decreased phosphorylation of MAPK and AKT KRAS, NRAS, and HRAS | KRAS            | Colon cancer                | 60        |

Continued
| Strategy | Target | Inhibitor | Result | RAS type | Cells/tissues | Reference |
|----------|--------|-----------|--------|----------|--------------|-----------|
| ICMT-1   | Knockout of ICMT | In vitro and in vivo inhibition of cell growth and oncogenic transformation | KRAS | Primary mouse embryonic fibroblasts | 45 |
| ICMT-1   | Cysmethynil | Mislocalization of RAS and impaired epidermal growth factor signaling lead to blocking of anchorage-independent growth | KRAS, NRAS, and HRAS | Mouse embryonic fibroblast | 61 |
| ICMT-1   | Knockout of ICMT | In vivo reduction of splenomegaly, immature myeloid cells in peripheral blood, and tissue infiltration by myeloid cells | KRAS | Myeloproliferative disorder | 46 |
| Ftase and GGTase | Allel knockout | Significant reduction in lung tumors and improved survival without apparent pulmonary toxicity | KRAS | Lung cancer | 44 |
| ICMT-1   | Cysmethynil or inhibitory RNA | Marked inhibition of tumor growth results from autophagy-induced apoptosis | Unknown | Liver and mouse embryonic fibroblast | 43 |
| Displace RAS from plasma membrane | Membrane-bound farnesyl-binding proteins | Salirasib | Reduction of the amount of RAS, disruption of serum-dependent and epidermal growth factor-stimulated ERK activation, inhibition of both anchorage-dependent and anchorage-independent growth, inhibition of tumor growth xenograft | KRAS | Pancreatic cancer | 62 |
| Membrane-bound farnesyl-binding proteins | Bryostatin-1 | Phosphorylation of KRAS and its dissociation, promotion of apoptosis, and reduction of in vitro and in vivo cell growth | KRAS | Jurkat T cells and NIH 3T3 cells | 63 |
| Membrane-bound farnesyl-binding proteins | Salirasib and Gemcitabine | Tumor growth inhibition among xenografts, reduction of KRAS, pAKT, and pMAPK, and decrease in total RAS level of liver biopsies | KRAS | Pancreatic cancer | 47 |
| Plasma membrane | Fendiline | Redistribution of KRAS from plasma membrane and inhibition of downstream signaling pathways | KRAS | Pancreatic, endometrial, lung, and colon cancer | 41, 48 |
inhibitor compounds overcome limitations associated with RAF dimerization. Next generation inhibitors take two approaches to combat RAF dimerization. The first approach is the development of compounds with the equal potency for inhibition of both monomeric and dimeric RAF. The second strategy is the recruitment of ATP binding cleft to disrupt RAF dimerization. Other than these therapeutic strategies, progress has been made in generating alternative agents to inhibit KRAS onc-RAF interaction which is needed to stimulate RAS-dependent oncogenic signaling. While targeted therapy against many cancers, such as EGFR-mutated cancers, provides effective responses, no FDA-approved KRAS onc-targeted therapy is currently available, and cytotoxic chemotherapy remains the best option for patients with KRAS onc-driven cancers. Hopefully, following the earlier failures in the direct inhibition of KRAS onc, a new wave of research in recent years has provided promising results. The KRAS oncoprotein has some specific structural features in comparison to wild-type KRAS. Selective targeting of these differences allows direct inhibition of the KRAS mutant without affecting wild-type KRAS. For example, recent studies focusing on the KRAS-G12C mutation as a direct inhibition strategy have been showed significant results. In this type of mutation, the thiol group of the cysteine residue located close to the nucleotide-binding pocket, switch I, and switch II, are targeted by different small molecules that result in the inhibition of downstream interactions. Since KRAS-G12C is the most common mutation in lung cancer patients, the translation of this agent to clinical practice would be a significant approach for generating novel anti-KRAS onc therapeutics.

RNA interference

The KRAS oncogene activates multiple downstream cellular pathways to drive the progression of cancer. Because of
| General mechanism of inhibition | Specific mechanism of inhibition | Inhibitor | Result | Reference |
|--------------------------------|--------------------------------|-----------|--------|-----------|
| Decreasing the proportion of RAS in GTP state | Inhibition of nucleotide exchange process without displacing of GDP | SCH 53870 | Inhibition of nerve growth factor -stimulated neurite outgrowth | 79 |
| | Impairing the nucleotide exchange and acceleration of the RAS GTPase activity | Sulindac sulfide | Decreases the RAS induced activation of the CRAF1 kinase | 80 |
| | Stimulation of GTPase activity of mutant RAS | GTP analogue | DABP-GTP restore GTPase activity of mutant KRAS | 71 |
| | Inactivation of KRAS in the GTP state | Calmodulin | Induction of ERK1/2 by calmodulin inhibition | 81 |
| | Inhibitory activity on intrinsic GEF-mediated nucleotide exchange | Arabinose-derived bicyclic compound | Mild selective toxicity effect on cells expressing oncogenic RAS-G13D | 82 |
| | Interfering with RAS-SOS interaction | Synthetic α-helix of SOS1 | Downregulation of RAS signaling | 83 |
| | Blocking the interaction of RAS as a substrate of SOS | DCAI | DCAI blocks the SOS-mediated nucleotide release and inhibits the activation of RAS | 84 |
| | Inhibition of SOS-catalyzed activation of KRAS | Multiple chemotypes including indoles, phenols, and sulfonamides and their analogues | Blocking binding of KRAS to SOS, and complete inhibition of nucleotide exchange | 85 |
| | Blocking GDP-GTP exchange | Andrographolide | Reduction in MAPK activation | 86 |
| | Prevention of GTP loading | SML-10-70-1 | Covalent labeling of KRAS, occupation of guanine nucleotide binding site, attenuation of AKT and ERK phosphorylation, and antiproliferative effect on different cell lines | 87 |
| | Subverting the native nucleotide preference to favour GDP over GTP | 6H05 fragment of tethering compounds | Impairing binding to RAF | 76 |
| | Prevention of GDP exchange by complete inhibition of KRAS-SOS complex | Maleimides | Significant inhibition of the RAS-RAF interaction | 73 |
| | Blockage of nucleotide association | Alpha helices of SOS1 (SAH-SOS1) | Downregulation of the MAPK signaling cascade | 72 |
| | Trapping drug-bound KRAS-G12C to its inactive state | ARS-853 | Decreased phosphorylation of CRAF, ERK (extracellular signal-regulated kinase), and AKT | 40 |
| | Disruption of the SOS1-KRAS interaction and thereby stabilization of the inactive GDP-bound conformation of KRAS | Ribonuclease binase | Inhibition of MAPK/ERK signaling | 68 |

Continued
the unsuccessful EGFR targeted therapy for KRASonc-dependent cancers and the difficulty associated with targeting KRASonc directly, a great deal of effort has been applied to target downstream effector pathways. The specific interaction of RAS family proteins with downstream effectors regulates various cellular functions\textsuperscript{3,77,96,97}. Constitutive activation of downstream effector pathways by oncogenic KRAS results in the uncontrolled growth, proliferation, and survival of cancer cells\textsuperscript{98}. It is essential to identify the effector pathways that are required for KRAS-driven carcinogenesis to identify pathways that should be targeted for treatment\textsuperscript{99}.

Two of the best-characterized KRAS effector pathways are the RAF-MEK-ERK and PI3K-AKT-mTOR pathways, which are integral to KRASonc-driven transformation through different signaling cascades\textsuperscript{100-102}. These pathways comprise different kinases, providing multiple nodes for potential therapeutic intervention\textsuperscript{103,104}. Collectively, studies on targeting the RAF-MEK-ERK and PI3K-AKT-mTOR pathways are divided into two categories. The first series of the studies focused on the identification of compounds

| General mechanism of inhibition | Specific mechanism of inhibition | Inhibitor | Result | Reference |
|--------------------------------|---------------------------------|-----------|--------|-----------|
| Interfering with GDP release through either inhibition of intrinsic or extrinsic catalyzed exchange mechanisms |  | ARS-853 | Significant loss of KRAS–CRAF interactions, inhibition of MAPK (including pMEK, pERK, and pRSK) and PI3K signaling (pAKT) pathways, loss of Cyclin D1 and Rb expression, an increase in the cell-cycle inhibitor p27, and an increase in hallmarks of apoptosis like PARP (Poly ADP-ribose polymerase) and sub-diploid DNA | 67 |
| Blocking the interaction of K-RAS-G12D with guanine nucleotide exchange factors |  | KRpep-2d peptide | Induction of large conformational changes in the Switch I and Switch II regions and significant inhibition of RAS activation | 74 |
| Disrupting RAS–effector interactions | Inhibition of the interaction between HRAS and RAF1 | MCP compounds | Reversion of RAS-transformed phenotype, inhibition of RAS-induced RAF1 activation, and MEK1 | 88 |
| Inhibition of RAS–RAF interaction |  | Sulindac derivative IND12 | Restoring epithelial morphology in malignantly transformed MDCK-f3 cells, and inhibition of cell invasion | 89 |
| Inhibition of RAS-CRAF interaction |  | Non-steroidal anti-inflammatory drug NS398 | Inhibition of up-regulation of MAP kinase phosphatases to suppress the ERK-mediated signaling | 90 |
| Inhibition of the interaction of RAS with the RAF-RAS binding domain |  | MCP compounds | Decreasing active, phosphorylated ERK1/2 | 91 |
| Stabilization of a protein conformation that has a weak affinity for effectors |  | Zn\textsuperscript{2+} cyclen | Inhibition of RAS-RAF interaction | 92 |
| Inhibition the binding of RAS–binding domain of RAF kinases to the RAS |  | Kobe0065 and its analog Kobe2602 | Downregulation of MEK/ERK, AKT, RALA, SOS, and induction of apoptosis | 93 |
| Inhibition of HRAS-GTP and CRAF1 binding |  | Rigosertib | Disruption of RAF activation, and inhibition of the RAS-RAF-MEK pathway | 78 |
targeting only one of the downstream signaling pathways, including RAF inhibitors, MEK inhibitors, or PI3K inhibitors (Table 3).

The results of these studies have shown that, due to the interplay between downstream signaling pathways of KRAS, inhibition of one downstream target leads to the overexpression of its interconnected pathways, creating a drug-resistant phenotype. For example, in response to MEK inhibition, PI3K is activated through a negative MEK-epidermal growth factor receptor-PI3K feedback loop\(^ {32,112} \). Therefore, novel therapeutic approaches are focusing on the disruption of these multiple nodes, which is only possible through the inhibition of multiple downstream kinases, rather than only one through combination therapy\(^ {100-104} \) (Table 4).

According to the valuable results from the combination therapy, extensive studies are moving forward based on multi-targeted therapy for the inhibition of KRAS\(^ {onc} \) downstream signaling pathways. Recently, a large trial investigated the therapeutic effects of the MEK inhibitor selumetinib and docetaxel in comparison to docetaxel alone, producing results in NSCLC patients with the KRAS mutation\(^ {114} \). Other results from an ongoing trial show a clinical benefit from combination therapy with an investigational MEK inhibitor known as PD-0325901 and palbociclib, an inhibitor of CDK4/6 (PD-0332991), in patients with KRAS-mutant NSCLC (NCT03170206) and KRAS-mutant PDAC (NCT03454035). In addition, phase II of the other ongoing study on investigational drugs GSK2256098 (focal adhesion kinase inhibitor) and trametinib (MEK inhibitor) was planned to evaluate the antitumor activity of this combination therapy in patients with advanced pancreatic cancer (NCT02428270). BVD-523, an ERK inhibitor, is also currently being tested in combination with nab-paclitaxel plus gemcitabine in a phase Ib trial in patients with metastatic pancreatic cancer (NCT02608229). Another downstream inhibitor is mTOR, a component of the PI3K pathway. The mTOR inhibitor (NCT02329717) PBI-05204 has been tested in patients with stage IV pancreatic cancer. In the other clinical trial, the pan-RAF inhibitor (LXH254) and ERK suppressor (LTT462) are being evaluated as combination therapy for patients with advanced-stage solid tumors with mitogen-activated-protein kinase (MAPK) alterations, including KRAS-mutant NSCLC (NCT02607813 and NCT02974725). Additionally, phase I/II trials have been initiated to assess the combination therapy of the MEK inhibitor trametinib and the BCL-XL and/or BCL-2 inhibitor navitoclax in patients with KRAS-mutant advanced-stage solid tumors (NCT02079740).

### Response evaluation criteria in solid tumors

RNA interference (RNAi) is based on a natural process by which RNA molecules inhibit the generation of protein from DNA\(^ {115,116} \). For example, in the search for novel strategies in the treatment of KRAS\(^ {onc} \)-driven cancer, microRNAs (miRs) have received attention for their role in the regulation of gene

---

**Table 3** Targeting downstream signaling pathways of RAS as therapeutic strategy

| Targets            | Inhibitor     | Results                                                                 | RAS proteins | Cancers                      | Reference |
|--------------------|---------------|-------------------------------------------------------------------------|--------------|------------------------------|-----------|
| RAF kinase         | BAY 43-9006   | Inhibition of tumor cell proliferation and tumor angiogenesis           | KRAS         | Colon, pancreatic, and breast cancer | 105       |
| MEK                | Selumetinib (AZD6244) with Docetaxel | Tumor volume change in mice with KRAS and p53 mutations, but resistance to combination therapy for mice with KRAS and LKB1 mutations | KRAS         | Lung cancer                  | 106       |
| MEK                | Selumetinib (AZD6244; ARRY-142886) | Pronounced G0/G1 arrest                                                 | KRAS and NRAS | NSCLC                        | 107       |
| MEK1/2             | Selumetinib with Temozolomide | Enhanced DNA damage and tumor growth inhibition                          | Unknown      | Colorectal cancer            | 108       |
| MEK                | Selumetinib (AZD6244) with Docetaxel | Improved median overall survival, median progression-free survival, and objective response | KRAS         | NSCLC                        | 109       |
| MEK                | Selumetinib and Trametinib | Reduction of tumor growth                                                | KRAS         | Lung cancer                  | 110       |
| p110α subunit of PI3K | SiRNA and/or BYL719 | Reduction of cell viability, induction of apoptosis, and cell cycle arrest | KRAS         | Colorectal cancer            | 111       |
expression. MiRs are small, single-stranded, highly conserved non-coding RNA molecules that are involved in the control of gene expression. These molecules exert their action by binding to target mRNAs to prevent protein production. The degree and nature of the complementarity between the microRNA and target mRNA determines the gene silencing mechanism that will be employed. Perfect complementarity to the mRNA target leads to its subsequent degradation and transcriptional inhibition, while partial complementarity results in the blockade of translation. Therefore, this complementarity plays a key role in regulating the target gene of a particular microRNA. For instance, polymorphisms of the let-7 microRNA binding site in the 3' untranslated region of KRAS leads to an impairment of their complementarity and elevated expression of KRAS.

The dysregulation of microRNAs and their critical roles in carcinogenesis results from the ability of microRNAs to control the expression of oncogenes and tumor suppressor genes. For a microRNA with tumor-suppressor activity, its downregulation promotes tumorigenesis, while overexpression of a microRNA with oncogenic effects leads to cancer development. Mechanisms responsible for the deregulation of miRs in cancers can be classified as genetic and epigenetic alterations that are observed in cancer cells. Considering KRAS as a proto-oncogene, downregulation of miRNAs that suppress KRAS activation and activation of miRNAs that modulate KRAS expression can lead to cancer development. Some of the microRNAs directly target KRAS, and some of them suppress KRAS activation through other targets (Table 5). For example, the results of a study showed that KRAS onc suppresses mir-200 family expression through its downstream effectors JUN and SP-1.

An alternative RNA therapeutic approach to miRNAs is through the use of small interfering RNAs (siRNAs) and short hairpin RNAs (shRNAs) from the family of non-coding RNAs. SiRNAs also regulate gene expression through gene silencing with inhibition of gene translation into protein. Additionally, their similarity in structural characteristics and pharmacokinetic profiles facilitates their use as therapeutics.

Regarding RNA-mediated silencing method, there are two strategies to suppress KRAS oncogenic signaling. First strategy is direct in which KRAS gene expression is reduced by direct binding of RNAi to KRAS onc mRNA (Table 5).
| RNA therapeutics | Direct target of inhibitory RNA | Clinical results | Cancers/Cell lines | Reference |
|------------------|---------------------------------|------------------|------------------|-----------|
| SiRNA            | KRAS                            | Inhibition of growth in metastatic and remetastatic cells as well as in primary tumor cells | Pancreatic cancer | 128       |
| SiRNA            | KRAS                            | Dramatic reversion of the transformed phenotype, reduction of subcutaneous tumor formation, increase in lag time and noninvasive tumor growth | Colorectal cancer | 129       |
| MiR-Let-7        | c-MYC                           | Significant growth suppression after treatment with miR-let-7a-1 precursor | Colon cancer | 130       |
| MiR-143          | KRAS                            | Inhibition of cell proliferation by over expression of micro-143 | Colon cancer | 131       |
| MiR-Let-7g       | KRAS                            | Increase in sensitivity to ionizing radiation after injection of miR-Let-7a | Lung cancer | 132       |
| MiR-18a          | KRAS                            | MiR-18a** repression leads to increased cell proliferation and promoted anchorage-independent growth | Squamous, colon, and hepatic cancer | 133       |
| MiR-Let-7        | RAS and c-MYC                   | Suppression of proliferation and induction of apoptosis through transfection with miR-Let-7a | Laryngeal cancer | 134       |
| MiR-Let-7a       | KRAS and c-MYC                  | Significant depression in tumor xenograft weight after injection of miR-Let-7a | Lung cancer | 135       |
| MiR-Let-7b and MiR-Let-7e | KRAS | Downregulation of miR-Let-7b and miR-Let-7e leads to increased resistance to cetuximab | Colorectal cancer | 136       |
| MiR-96           | KRAS                            | Transfection with pre-miR-96 results in reduction of cell growth, cell migration, and strong invasive capacity of cells | Pancreatic cancer | 137       |
| MiR-181a         | KRAS                            | Ectopic expression of miR-181a leads to suppression of cell proliferation and anchorage-independent growth ability | Oral squamous cancer | 138       |
| MiR-30c          | KRAS                            | Overexpression of miR-30c resulting in inhibition of cell proliferation | Breast cancer | 139       |
| MiR-Let-7a       | KRAS                            | Chemoradiation therapy resistance after inhibition of miR-let-7a | Colorectal cancer | 140       |
| MiR-143 and MiR-145 | CD44, KLF5, KRAS, and BRAF | Reduction of cell proliferation, migration and chemoresistance by restoring miR-143 and miR-145 | Colon cancer | 141       |
| SiRNA            | KRAS                            | Significant inhibition of proliferation and EMT, and tumor growth and prolonged mouse survival | Pancreatic cancer | 142       |
| MiR-Let-7g       | KRAS and HMGA2                  | Significant inhibition of cell proliferation, migration, and invasion following overexpression of miR-Let-7g | Hepatocellular cancer | 143       |
| MiR-193b/365a cluster | KRAS and MAX                  | Inhibition of cell proliferation, clonogenic potential, and migration with ectopic expression of miR-193b/365a cluster | Cutaneous squamous cancer | 144       |
| MiR-30b          | KRAS, PIK3CD, and BCL2          | Suppression of cell proliferation and tumor growth following overexpression of miR-30b | Colorectal cancer | 145       |
| MiR-96           | Ecotropic viral integration site 1 (EVI1) and KRAS | Inhibition of miR-96 leads to attenuation of growth inhibition | Pancreatic ductal cancer | 146       |

Continued
Second approach is indirect, which is based on the inhibition of synthetic lethal interactions. Synthetic lethality is a phenomenon through which a genetic alteration leads to cell death only in the presence of another genetic perturbation. Mechanistically, synthetic lethal interactions can involve genes that are functionally connected\textsuperscript{149}. In cancer cells, aside from pathways directly controlled by oncogenes, there are several non-oncogene-targeted pathways, which are involved in the process of transformation\textsuperscript{149}. Thus, oncogenes require additional support from other genes to maintain the oncogenic state\textsuperscript{26}. A major challenge in cancer treatment is the identification of targets that can be inhibited for the selective killing of cancer cells while sparing normal cells. Synthetic lethal interactions between oncogenes and non-oncogenes in cancer cells increase the sensitivity of cancer cells to selective therapeutics in comparison to normal cells\textsuperscript{150}.

The KRAS mutation predisposes cancer cells to additional dependencies on the activity of genes that are not directly regulated by KRAS. This phenomenon can provide an approach for the selective treatment of KRAS\textsuperscript{onc}-driven cancers according to the synthetic lethal interactions\textsuperscript{26,151}. The KRAS signaling pathway is complex, so several potential synthetic lethal targets are required for the initiation or maintenance of KRAS mutant tumors. To identify critical nodes in the signaling pathways regulating aberrant KRAS\textsuperscript{onc} signaling, RNA silencing technologies could be exploited\textsuperscript{152}. These newly identified synthetic lethal interactions lead to novel therapeutic opportunities. Additionally, small-molecule synthetic lethality screens have resulted in the identification of the selective effect against KRAS mutant cells compared with wild-type cells\textsuperscript{31,153}. It should be noted that inhibition of synthetic lethal interactions is not only accomplished by RNAi, but also with small molecules. A summary of researches on the screening of synthetic lethal interactions with KRAS\textsuperscript{onc} through RNA silencing methods or small molecules is provided in Table 6.

Antisense oligonucleotides directed against KRAS\textsuperscript{onc} have indicated a therapeutic benefit in laboratory studies, opens up multiple effective possibilities for suppressing KRAS activity, and preventing the feedback response and drug resistance while facilitating combination therapy\textsuperscript{99}. Despite the tremendous potential of RNA-based therapies, the successful application of this technology is currently limited. RNAs are inherently unstable, and therefore there is lack of efficient delivery of sufficient amounts to the target tissue. Additionally, toxicity due to off-target effects and the induction of immune system responses also represent difficulties related to this approach\textsuperscript{115,158,159}.

**Targeting the immune system**

Cancer immunotherapy for patients carrying the KRAS mutation has become a clinical oncology reality. KRAS-G12D knockout cells show increased production of interleukin 18 by the host immune system, leading to a dramatic reversion of the transformed phenotype and reduction of the proliferation rate of cancer cells\textsuperscript{129}. Increases in NK cells and

| RNA therapeutics | Direct target of inhibitory RNA | Clinical results | Cancers/Cell lines | Reference |
|------------------|--------------------------------|------------------|-------------------|-----------|
| SiRNA            | KRAS                           | Decrease in cell number and significant inhibition of tumor growth | Lung cancer | 127       |
| SiRNA            | KRAS                           | Decrease in cell viability and proliferation, induction of apoptosis, and attenuation of tumor growth through inhibition of the MAPK pathway | Colorectal cancer | 99        |
| MiR-134          | KRAS and STAT5B                | Inhibition of cell proliferation, induction of apoptosis, cell death, and xenograft tumor growth suppression through overexpression of miR-134 | Glioblastoma | 147       |
| MiR-1            | KRAS and MALTA-1               | Inhibition of cell proliferation, increased apoptosis \textit{in vitro}, reduction of tumor growth, and metastasis by overexpression of miR-1 | Breast cancer | 30        |
| MiR-134          | KRAS                           | Inhibition of proliferation and growth with promotion of apoptosis and sensitivity to the drug following overexpression of miR-134 | Gastric cancer | 118       |

\textsuperscript{1}Pre-miRNA is further cleaved to generate mature miRNA and antisense miRNA star products (miRNA\textsuperscript{*}).
antibody-dependent cell-mediated toxicity after combination therapy with lenalidomide and cetuximab lead to increases in circulating naïve and central memory T cells in patients with KRAS-mutant colorectal cancer\textsuperscript{160}. The KRAS mutation induces increased expression of programmed cell death 1 ligand 1 (PD-L1)\textsuperscript{161}. According to these findings, Ebert et al.

| Inhibitor | Cancer cell | Target | Conclusion | Reference |
|-----------|-------------|--------|------------|-----------|
| Oligonucleotide-directed mutagenesis | NIH 3T3 fibroblasts | RAC/RHO pathway | Impairment of RAS-mediated transformation | 154 |
| SiRNA | Human lung cells | PKC | Apoptosis induction and suppression of the growth of KRAS mutant human lung tumor xenografts | 155 |
| ShRNA | Human lung epithelial cells | TBK1 | In vitro: reduction of cell viability. In vivo: inhibition of growth of tumor xenografts and induction of apoptosis | 152 |
| ShRNA | Colorectal cancer cell lines | THOC1 | Reduction of mutant cell fitness percentage | 26 |
| ShRNA | Colorectal cancer cell lines | COP1 | Impaired growth on adherent surfaces | 26 |
| Bl-2536 and shRNA | Colorectal cancer cell lines | PLK1 | Increased toxicity towards RAS mutant cells and reduction of cell fitness percentage | 26 |
| MiR-Let-7 | Colon cancer-MYC | c-MYC | Significant growth suppression after treatment with miR-let-7a-1 precursor | 130 |
| ShRNA, MG132, and Bortezomib (Velcade) | Colorectal cancer cell line | Anaphase promoting complex (APC) subunits | Reduction of mutant cell fitness percentage and G2/M arrest | 26 |
| ShRNA | Murine embryonic fibroblasts | ATR-CHK1 pathway | Suppression of proliferation due to the synergistic increases in genomic instability | 31 |
| ShRNA | Human NSCLC cell lines | Wilms tumor 1 (WT1) | Induction of senescence and decrease of proliferation | 156 |
| ShRNA and siRNA | Colon cancer | Snail2 | Impaired colony formation in soft agar and suppressing the malignant phenotype by reversion of EMT | 157 |
| SiRNA and Bortezomib | Human colon cancer cell line: HCT-116 | CDC6 and proteasome | Induction of apoptosis | 149 |
| MG-132 and proteasome inhibitor I | Human colon cancer cell line: HCT-116 | Proteasome | Pro-apoptotic and loss of viability responses | 149 |
| Bortezomib, Topotecan, and Doxorubicin | Human colon cancer cell line: HCT-116 | Proteasome and topoisomerase | G2/M arrest | 149 |
| SiRNA and Bortezomib with Fasudil | NSCLC cell lines | Proteasome components, IL-1 signaling, and Rho-signaling pathways, regulated by GATA2 | Reduction of mutant cells viability, tumor burden, tumor number, and average tumor size | 150 |
| MIR-200 family | Lung and breast cancer | BCL2 | Restoration of mir-200 resulting compromised KRAS-induced cellular transformation, apoptosis, EMT transition, and tumor formation | 119 |
| ABT-263 and Selumetinib | Colorectal, lung, and pancreatic cancer | BCL-XL and MEK | Promotion of apoptosis | 151 |
| Navitoclax, G-963, and GDC-0941 | NSCLC and pancreatic cancer | BCL2/BCL-XL, MEK, and PI3K | Suppression of AKT activation resulting in increased cytotoxicity, cell population with sub-2N DNA content, and PARP\textsuperscript{‡} cleavage | 153 |

\textsuperscript{‡} Poly (ADP-ribose) polymerase
showed that anti-PD-L1 antibodies significantly reduced tumor size in MEK inhibitor-treated mice with the KRAS mutation. Thus, it seems that the combination of immunotherapy and anti-proliferative agents, such as MEK inhibitors, provides higher anti-tumor activity\textsuperscript{162}. Genetic alterations are specific to cancer cells and are not present in normal cells; thus, treatments that specifically target the protein product of these genetic aberrations may provide a clinical benefit in the absence of normal cell toxicities. Although mutant KRAS proteins themselves are not strongly immunogenic, efforts are underway to enhance the ability of the immune system to recognize KRAS mutant peptides as neo-epitopes. For example, specific immunogenic mutations could help to recognize KRAS mutant variant peptides of the most frequent KRAS mutations, such as G12V and G12D, by specific T cell receptors\textsuperscript{163}. In this way, to develop more effective personalized immuno-therapy for patients with the KRAS mutation, Rosenberg’s team isolated tumor-infiltrating lymphocytes (TILs) with the ability to specifically target the KRAS mutation. The findings of that study, which were presented in December 2016, introduced, for the first time, a novel immunotherapy-based strategy, called adaptive T cell transfer immunotherapy. These results validated the possibility of using personalized T cell receptor gene therapy against multiple types of cancer expressing this common mutation or other types of KRAS mutations\textsuperscript{163}.

Thus, the purpose of recent studies has been the identification of immune-editing of T cells during tumor development, as well as the determination of their potential applications for tumor-specific immunotherapy\textsuperscript{164}. According to the brilliant results from immunotherapy, treatments focused on altering the immune system for patients suffering from KRAS\textsuperscript{onc}-driven cancers have been intensively investigated in recent years, with new achievements. In one study, the efficacy of immune checkpoint inhibitors among NSCLCL patients was found to correlate with the KRAS mutation as a molecular smoking signature\textsuperscript{165}. Other evidence indicates that the co-mutation of TP53 and KRAS in lung adenocarcinoma can be exploited as a potential predictive marker for effective immune checkpoint blockade immunotherapy\textsuperscript{164}. Clinical trials have also been initiated for the KRAS-G12D-specific cancer vaccine TG01/ GM-CSF either alone or combined with gemcitabine. The initial results of these trials have shown an induction of the immune responses in response to TG01/GM-CSF plus gemcitabine combination therapy\textsuperscript{166}. A study to evaluate the efficacy and safety of cobimetinib plus atezolizumab and atezolizumab monotherapy versus regorafenib in participants with metastatic colorectal adenocarcinoma is currently ongoing as a phase III trial (NCT02788279). The initial findings suggest that this therapeutic strategy is helpful in improving the immune response. One trial examining the combination therapy of a newer CDK4/6 inhibitor, abemaciclib, with the immune checkpoint inhibitor pembrolizumab is currently ongoing in NSCLC patients with the KRAS mutation\textsuperscript{167}. New achievements have been observed in these studies against human cancers (Table 7), represent the need for further studies to enhance immunotherapeutic efficacy in some patients.

**Other approaches**

Despite important strides made in the development of targeted therapy for KRAS\textsuperscript{onc}-mediated cancers, no therapeutic approaches are clinically available. In recent years, a deeper understanding of the critical parameters involved in the promotion of KRAS\textsuperscript{onc}-driven tumorigenesis has been considered for the development of new therapeutic options. In this part of the article, we review these new achievements and discuss multiple lines of evidence of novel key pathways that are recognized to interact with other previously identified KRAS-regulated survival pathways to transduce signals of carcinogenesis. The data suggest that co-targeting of these newly and previously recognized KRAS\textsuperscript{onc} regulated pathways has significant clinical potential.

**Inhibition of stem cell program**

Cancer stem cells (CSCs) are defined as tumor-initiating cells with self-renewal capacity. They are considered to be responsible for cancer initiation, progression, metastasis, drug resistance, and treatment relapse\textsuperscript{168}. The KRAS mutation has been shown to preferentially alter the profile of gene expression to induce embryonic stem cell-like features\textsuperscript{169}. For example, the expression of some genes is known to be upregulated in the presence of the KRAS mutation, including fibroblast growth factor receptor 1 (FGFR1), which plays a common role in both embryonic and cancer development, LCK, the transcriptional silencing of which is required for embryonic stem cell differentiation, and the induced-pluripotency factor SOX2, which reprograms differentiated cells to pluripotency. In contrast, KLF4 expression was suppressed in KRAS mutant colon cancer cells, which is consistent with its induction of multiple cell lineage differentiation in the intestine\textsuperscript{18}. Additionally, a KRAS-centric mechanism would apply in the context of epidermal-mesenchymal transition (EMT) to generate CSCs
through the WNT pathway\textsuperscript{170}.

Other results have indicated that oncogenic KRAS activation in the genetic background of loss-of-function of adenomatous polyposis coli (APC) results in enhanced CSC activation by increasing both intracellular stabilization of $\beta$-catenin and the MAPK pathway\textsuperscript{171,172}. Furthermore, endodermal progenitors expressing KRAS-G12V do not differentiate upon retinoic acid treatment and continue to proliferate and maintain stem cell characteristics\textsuperscript{173}. Several studies have described the KRAS mutation as a driver of stem cell-like properties of cancer cells. Thus, inhibition of multiple key pathways involved in embryonic stem cell signaling represents a novel therapeutic strategy. Le Rolle et al.\textsuperscript{18} showed that inhibition of KRAS mutant colon tumors with miR145, an epigenetic regulator and an embryonic stem cell inhibitor, suppressed their malignant growth. Data suggest that salinomycin, the most potent cancer stem cell inhibitor with potential efficacy in human cancers, specifically disrupts KRAS\textsuperscript{onc} nanoscale membrane organization, effectively reducing effector recruitment to KRAS\textsuperscript{onc}, which then compromised at least MAPK signaling and proliferation\textsuperscript{170}. Ophiobolin A, another candidate CSC drug, has been found to possess higher potency than salinomycin and exert its KRAS4B-specific activity through the inactivation of calmodulin\textsuperscript{170}.

Based on the role of the KRAS\textsuperscript{onc} in stemness, $\alpha$-Mangostin-encapsulated PLGA [poly (D, L-lactic-co-glycolic acid)] nanoparticles show inhibitory effects on carcinogenesis in transgenic mice carrying the KRAS mutant allele through the downregulation of pluripotency maintenance factors (c-MYC, NANOG and OCT4) and stem cell markers (CD24 and CD133)\textsuperscript{174}. Overall, these data suggest that targeting multiple signaling pathways of cancer stem cell activation induced by the KRAS mutation could be an attractive therapeutic approach.

### Targeting receptor tyrosine kinases (RTKs)

A growing body of evidence suggests that the KRAS mutation may serve as a predictive resistance marker to guide the use of anti-EGFR therapy. Multiple studies have demonstrated that patients with mutations in KRAS do not appear to experience a clinical benefit from anti-EGFR monoclonal antibody treatment\textsuperscript{175}. In cancers with KRAS mutations, part of the cell survival and proliferation pathways could still be due to the activation of upstream RTKs other than EGFRs. Therefore, another possible approach to target tumors with KRAS mutations is through the inhibition of such critical RTKs that contribute to the enhanced prosurvival. The type 1 insulin-like growth factor receptor is a promising target in different types of cancers, including colon cancer\textsuperscript{176}. The PI3K signaling pathway is a common downstream effector of both IGF-1R and KRAS. Thus, blockade of IGF-1R using different monoclonal antibodies or tyrosine kinase inhibitors is theoretically relevant for the treatment of patients with KRAS\textsuperscript{onc}-driven cancers\textsuperscript{8}. Although patients with the KRAS mutation show resistance to EGFR-targeted therapy, preclinical data have indicated that combination therapy with

---

**Table 7**  Studies on immune system targeting RAS-driven cancers

| Immunomodulator | Mechanism of action | Results | Cell line | Reference |
|-----------------|---------------------|---------|-----------|-----------|
| Host immune system | KRAS\textsuperscript{G12D}, knockdown cells increased production of interleukin 18 by host immune system | Dramatic reversion of the transformed phenotype, reduction of proliferation rate subcutaneous tumor formation | KRAS\textsuperscript{G12D} murine C26 colorectal cancer cells | 129 |
| Lenalidomide in combination with cetuximab | Increase in NK cells and antibody dependent cell-mediated toxicity | Increases in circulating naive and central memory T cells | KRAS-mutant metastatic colorectal cancer cells | 160 |
| Engineered T cells | Activity of T-cell receptors of engineered T cells against the HLA-A’11:01* tumor lines presenting mutated KRAS variants | Reduction of tumor growth in xenograft model | KRAS mutant human pancreatic tumor lines | 163 |
| MEK inhibition in combination with anti-PD-L1 | Induction of the accumulation of antigen-specific CD8$^+$ T cell effectors in tumors and prevention of the "exhaustive" T cell death | Durable tumor regression | CT26 colon carcinoma cell line harboring mutant KRAS\textsuperscript{G12D} | 162 |
| Pembrolizumab | PD-1 blockade immunotherapy | Remarkable clinical benefit to PD-1 inhibitors | Lung adenocarcinoma | 164 |
IGF-1R and EGFR kinase inhibitors results in synergistic growth inhibition in colorectal cancer cell lines. Hurwitz et al. showed a clinical benefit following the treatment of patients with bevacizumab as an anti-vascular endothelial growth factor (VEGF) therapy. Data have also shown that, unlike anti-EGFR therapy, anti-VEGF therapy functions independently of the KRAS mutation status, revealing even greater clinical significance.

**Stabilization of the G-quadruplex**

G-quadruplexes (G4) are special secondary structures containing runs of guanines separated by other bases. The localization of G4 in the human genome was found to be non-random, indicating their important role in the regulation of functional regions. Significantly, G4 are more frequent in oncogenes or regulatory genes than in house-keeping or tumor suppressor genes. Their higher distribution in the promoters of oncogenes suggests a possible involvement of G4 in cancer. Genome-wide analysis of human cells has revealed the role of these structures is gene-silencing through the inhibition of replication, transcription, and translation. Therefore, the stabilization of guanine-rich regions located in the oncogene promoters represents a highly valuable new molecular target for the development of novel anti-cancer therapeutics. It is now evident that the core promoter region of KRAS contains silencing G4 elements. G-to-T knockout mutations in the G4-forming regions of the KRAS promoter were found to disrupt or abrogate G4 formation. In addition, stabilization of the KRAS promoter by the cationic porphyrin TMPyP4 leads to a significant decrease in KRAS expression. The interaction of G4 of the KRAS promoter with natural polyphenols, such as ellagic acid and curcumin, has also been confirmed by UV-vis spectroscopy. Significantly, the melting temperature of the G-quadruplex is increased, indicating its stabilization upon interaction with polyphenol ligands.

**Inhibition of inflammation**

KRAS-driven tumorigenesis is tightly connected with tumor-promoting inflammation, which increasingly represents another promising therapeutic strategy. According to recent clinical data indicating the role of inflammation in the carcinogenesis related to the KRAS mutation, targeting inflammatory signaling pathways seems to be an essential component of therapy for tumors with KRAS mutations. Different cellular pathways, which are modulated by KRAS and induce inflammation, include JAK/STAT, NF-κB, MAPK, and immune checkpoint signaling pathways. For example, the KRAS mutation contributes persistent pancreatitis induced by cerulein. In this situation, suppression of inflammation by deletion of IKK-β and inhibition of NF-κB activity interferes with dysplasia. In contrast, overexpression of IKK-β cooperates with the KRAS mutant allele to promote oncogenesis.

A different study indicated that while persistent KRAS activation drives the secretion of STAT3 pathway mediators, activation of STAT3 results in the amplification of KRAS oncogene through the upregulation of anti-apoptotic and pro-proliferative proteins. Co-administration of azoxymethane (AOM) and dextran sodium sulfate (DSS), respectively, as carcinogenic and inflammatory agents, results in a significant decrease in the latency of KRAS-driven tumor formation. Given the presence of inflammatory stimuli in a KRAS mutation background as positive feedback promoting KRAS-associated carcinogenesis, targeting each of the mentioned signaling pathways would likely lead to the development of a mechanism for disease control.

**Targeting metabolic pathways**

Metabolic reprogramming of cancer cells due to oncogenic mutations is critical for cell growth and survival. Data show that the KRAS oncoprotein confers metabolic robustness for the acquisition of cellular metabolism networks to convert carbon sources into biomass. The metabolic features of KRAS-driven cancers can be explained through the reprogramming of glucose, amino acids, and lipid metabolisms. Cancer cells harboring KRAS promote the glycolytic switch, glucose uptake, increased channeling of glucose-derived metabolites into the tricarboxylic acid cycle, and activation of glucose-dependent biosynthetic pathways. For example, it has been reported that the KRAS mutation increases the expression of glucose transporter-1 (GLUT1) and several rate-limiting glycolytic enzymes. Interestingly, the induction of metabolic changes is dependent on the content of the KRAS mutant allele of cancer cells. Thus, glycolytic gene expression was markedly enhanced in KRAS-G12D/G12D relative to heterozygous lung tumor cells. One mechanism by which KRAS aberrantly regulates metabolic networks is through the reprogramming lipid metabolism by the promotion of cellular uptake, retention, accumulation, synthesis, and oxidation of fatty acids. For instance, lung cancer cells carrying the KRAS mutation are highly dependent on the activity of acyl-coenzyme A synthetase long-chain family member 3 (ACSL3). Mutated KRAS promotes...
lipogenesis through the induction of fatty acid synthase, leading to lipid signatures of human lung cancer cell lines\textsuperscript{189}. Other results have shown that the RAS mutation leads to the reprogramming of de novo lipogenesis of cancer cells by scavenging serum fatty acids\textsuperscript{190}. Emerging evidence from different research groups indicates that KRAS mutations are associated with changes in amino acid metabolism\textsuperscript{191}. Reprogramming of glutamine metabolism in KRAS\textsuperscript{onc}-driven cancers is the most important alteration in amino acid metabolism. While most cells utilize glutamate dehydrogenase 1 for conversion of glutamate into α-ketoglutarate, cancer cells carrying the KRAS mutation convert glutamate to aspartate\textsuperscript{191}. The increased requirement for branched-chain amino acids (BCAAs) is a very early phenomenon during tumor development, similar to some types of KRAS\textsuperscript{onc}-driven cancers\textsuperscript{192}. As mitochondrial activity is required for metabolic changes in cancer cells, autophagy as a mechanism for the elimination of defective mitochondria is crucial for tumor growth. Loss of essential autophagy genes in KRAS\textsuperscript{onc}-driven cancer impairs effective mitochondrial function and suppresses tumor progression, emphasizing the role of autophagy in the intracellular nutrient supply\textsuperscript{193}. These reports indicate that the KRAS mutation creates unique metabolic dependencies that could be exploited for anti-cancer therapy.

**Targeted RNA replacement**

Tetra hymena group I intron-based trans-splicing ribozyme is specific therapeutic tool with ability to discriminate the target RNA resulting in specific and high-fidelity cleavage reaction of its target\textsuperscript{194}. Moreover, ribozymes can specifically transfer the therapeutic gene into cancer cells expressing target RNA. This specific trans-splicing reaction with the ability of discrimination target RNA from non-target one, even with a single nucleotide difference, makes it as an attractive novel treatment strategy for KRAS point mutations. Regarding KRAS-G12V mutation as one of the most prevalent point mutation, Tetra hymena group I intron-based trans-splicing ribozyme designed for selective cleavage of KRAS-G12V transcript\textsuperscript{195}. An accurate and specific intracellular trans-splicing reaction of the designed ribozyme systems with the KRAS-G12V target RNA, leads to efficient reduction of transcript level. Except that replacement of RNA, concurrent induction of suicide gene activity resulting in cytotoxicity and effective retardation of cancer cells harboring KRAS mutation\textsuperscript{196}. Moreover, trans-splicing and therapeutic anti-cancer gene activity was selectively and efficiently induced only in KRAS-mutant cancer cells without targeting of cells expressing wild-type KRAS\textsuperscript{195}.

**Oncogene-induced senescence**

Oncogene-induced cellular senescence (OIS) is a complex mechanism of tumor suppression which is thought to be triggered by aberrant activation of oncogenic signaling\textsuperscript{197}. Undisputed role of RAS\textsuperscript{onc} in different human cancers, necessitate studies on the RAS\textsuperscript{onc}-induced senescence as an alternative treatment strategy. Senescence is not a simple mechanism triggered by only linear series of events and multiple components are required to establish a senescence response. Accordingly, detailed molecular mechanisms underlying OIS should be completely understood to provide adequate mechanistic insight for implementation of RAS aberrant oncogenic signaling against themselves as a potential anti-cancer strategy\textsuperscript{198}.

Basically, there are three pathways which are recruited by KRAS\textsuperscript{onc} to induce senescence which are also interconnected. The first pathway is transcriptional repression of pro-proliferative genes like E2F target genes. In addition to the transcriptional repression, a second pathway that is believed to mediate KRAS\textsuperscript{onc}-induced senescence is the DNA damage pathway. Oncogene activation induces aberrant DNA replication events, leading to replication stress and subsequent DNA damage\textsuperscript{198}. Consequently, DNA damage and accumulation of proteins involved in DNA damage response, like ATM and CHK2 results in senescence induced by oncogene activation. Finally, a third pathway, which is essential for senescence and recruited under RAS activation is senescence-associated secretory phenotype (SASP). Studies have recognized that SASP mediates RAS\textsuperscript{onc}-induced senescence, through the secretion of specific proteins like C/EBPβ transcription factor\textsuperscript{199}. Notably, the neurofibromatosis type 1 (NF1), encoding a RAS-specific GAP, has been implicated in OIS\textsuperscript{200}. In this context, suppression of Ras and/or PI3K are sufficient to induce senescence, and these events on their own can activate the known downstream mediators of the senescence response (Rb and p53) through a variety of mechanisms\textsuperscript{200} (Figure 1). Moreover, in BRAF-driven melanomagenesis, loss of NF1 cooperates with RAF mutations by increasing PI3K/AKT signaling and preventing entry into OIS\textsuperscript{201,202}. While the significant role of the oncogenic RAS in human cancers has been proved for many years, a better understanding of the molecular basis of RAS\textsuperscript{onc}-mediated senescence, allows the delineation of new therapeutic approaches surprisingly aimed at engagement of oncogenic signaling against oncogenic signaling.
Conclusions

More than 30 years of intensive research and tens of thousands of published studies have provided valuable insights into the biology, biochemistry and biophysics of RAS family proteins. Signal transduction of RAS (most notably KRAS) is regulated by three classes of canonical interacting partners, including regulators that control activation of the GTPase cycle (by GEFs), its inactivation (by GAPs), and a wide spectrum of effectors (e.g., RAF kinase and PI3 kinase) that initiate signaling cascades downstream of RAS and RAS-like proteins. We have gained deep knowledge about their membrane trafficking, structure-function relationship, mechanisms of GDP/GTP binding and accelerated nucleotide exchange by GEFs, intrinsic and GAP-stimulated GTP hydrolysis, interaction with effectors and activation of diverse signaling pathways. However, these studies have their own eligibility confinement: cell-free investigations have been predominantly carried out in the absence of lipid membrane, using defined domains rather than full-length proteins, and cell-based studies have mostly been performed via the heterologous expression of tagged genes and their variants in methodologically congenial cell lines. As the omics era is coming to an end and research has decelerated, many new movements have emerged, especially due to the accessibility of new technologies. Several novel mechanisms have been uncovered that have extended our understanding of the role of protein-protein/protein-lipid interactions and various types of post-translational modifications in the modulation of RAS protein activity. Another issue is the activation mechanism of regulators and effectors. Notably, the identification of additional components of the RAS interaction networks is a critical step towards understanding both the relationship between RAS proteins and the selective activation of respective effectors, as well as the molecular signatures required for the spatiotemporal integration and activation of GEFs and GAPs. The identification and functional reconstitution of specific interaction networks by using appropriate liposomes and full-length effector proteins may eventually provide fundamental insights into the functional characterization of multiprotein complexes of RAS and the complete identification of regulatory mechanisms. In this context, an interesting issue, which is increasingly appreciated, is a RAS-membrane interaction that appears to generate RAS isoform specificity with respect to regulator and effector interactions. Currently, it has become more evident that an increasing number of additional RAS binding partners are critical in modulating and integrating RAS in various signaling networks at biological membranes.

This phenomenon is likely achieved by scaffold proteins, including CAM, GAL1, GAL3, IQGAP1, NCL, NPM1, SHOC2, SPRY, SPRD1 and GAB1, which may modulate isoform specificity at specific sites of the cell. However, the roles of these additional RAS interaction proteins as novel modulators of RAS signaling remain unclear. Hence, elucidation of the RAS signal transduction requires not only RAS-effector interactions but also additional structures and the interplay of multi-protein complexes. Keeping this in mind, accumulating evidence supports a role for cell type-dependent RAS paralog functions that should prompt future efforts to examine the respective pathways in a more context-specific manner. Excluding driver mutations, passenger mutations accumulate and frequently escape natural negative selection, resulting in several oncological outcomes. In parallel with standard tumor profiling methods, high-throughput technologies, such as next-generation sequencing, have been employed to shift the treatment paradigms. Thus, further characterization of the heterogeneous identity of patient tumor tissue exploring all specific molecular aberrations along with the specific KRAS mutation, seems to be critical for an effective therapy. Such efforts could lead to the identification of disease-specific therapeutic opportunities. The other novel technology is phosphoprotein analysis through kinome profiling, which provides evidence of signaling pathways that are activated in a patient’s tumor.

The authors of this review article conclude that translating our knowledge of different treatment frameworks to the clinic via targeted therapy of the KRAS onc and personalized immune-therapy may be the best strategies to dramatically improve patient outcomes. In summary, we are at the beginning of a new series of attempts to treat KRAS onc-driven cancers by directly targeting the protein or through personalized targeted therapy with high-throughput or immunotherapy-based strategies. This new wave of personalized studies provide hope for thousands of patients suffering from KRAS onc-driven cancers.

Acknowledgments

Authors are thankful to Dr. Seyed Ali Jafari (Mashhad University of Medical Sciences) for insightful comments, and to Dr. Saeideh Nakhaei-Rad for her valuable suggestions. We thank American Journal Experts and Ms. Diana Inanlou for language edition of the manuscript. M.R.A. was supported by the European Network on Noonan Syndrome and Related Disorders (NSEuroNet, Grant No. 01GM1602B), and the German Federal Ministry of Education and Research.
(BMBF): German Network of RASopathy Research (GeNeRARe, Grant No. 01GM1519D & 01GM1902C).

**Conflict of interest statement**

No potential conflicts of interest are disclosed.

**References**

1. Simanshu DK, Nissley DV, McCormick F. RAS proteins and their regulators in human disease. Cell. 2017; 170: 17-33.
2. Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. J Clin Oncol. 2010; 28: 4697-705.
3. Nakachi-Rad S, Haghighi F, Nouri P, Rezaei Adariani S, Lissy J, Kazemein Jasemi NS, et al. Structural fingerprints, interactions, and signaling networks of RAS family proteins beyond RAS isoforms. Crit Rev Biochem Mol Biol. 2018; 53: 130-56.
4. Vasan N, Boyer JL, Herbst RS. A RAS renaissance: emerging targeted therapies for KRAS-mutated non-small cell lung cancer. Clin Cancer Res. 2014; 20: 3921-30.
5. Cox AD, Der CJ, Philips MR. Targeting RAS membrane association: back to the future for anti-RAS drug discovery? Clin Cancer Res. 2015; 21: 1819-27.
6. Martín-Gago P, Fansa EK, Wittinghofer A, Waldmann H. Structure-based development of PDE6 inhibitors. Biol Chem. 2017; 398: 535-45.
7. Narla G, Sangodkar J, Ryder CB. The impact of phosphatases on proliferative and survival signaling in cancer. Cell Mol Life Sci. 2018; 75: 2695-718.
8. Chakravarti A, Loeffler JS, Dyson NJ. Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. Cancer Res. 2002; 62: 200-7.
9. Hu YP, Patil SB, Panasiewicz M, Li WH, Hauser J, Humphrey LE, et al. Heterogeneity of receptor function in colon carcinoma cells determined by cross-talk between type I insulin-like growth factor receptor and epidermal growth factor receptor. Cancer Res. 2008; 68: 8004-13.
10. Hurwitz HI, Yi J, Ince W, Novotny WF, Rosen O. The clinical benefit of bevacizumab in metastatic colorectal cancer is independent of K-ras mutation status: analysis of a phase III study of bevacizumab with chemotherapy in previously untreated metastatic colorectal cancer. Oncolgy. 2009; 14: 22-8.
11. Chow HY, Jubb AM, Koch JN, Jaffer ZM, Stepanova D, Campbell DA, et al. p21-Activated kinase 1 is required for efficient tumor formation and progression in a Ras-mediated skin cancer model. Cancer Res. 2012; 72: 5966-75.
12. Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K pathway in human disease. Cell. 2017; 170: 605-35.
13. Ryan MB, Der CJ, Wang-Gillam A, Cox AD. Targeting RAS-mutant cancers: is ERK the key? Trends Cancer. 2015; 1: 183-98.
14. Yuan TL, Amzallag A, Bagni R, Yi M, Afghani S, Burgan W, et al. Differential effector engagement by oncogenic KRAS. Cell Rep. 2018; 22: 1889-902.
15. Zhu ZH, Aref AR, Cohoon TJ, Barbie TU, Imamura Y, Yang SH, et al. Inhibition of KRAS-driven tumorigenicity by interruption of an autocrine cytokine circuit. Cancer Discov. 2014; 4: 452-65.
16. de Ruiter ND, Burgering BMT, Bos JL. Regulation of the forkhead transcription factor AFX by Raf-dependent phosphorylation of threonines 447 and 451. Mol Cell Biol. 2001; 21: 8225-35.
17. Kerkhoff E, Rapp UR. Cell cycle targets of Ras/Raf signalling. Oncogene. 1998; 17: 1457-62.
18. Le Rolle AF, Chiu TK, Zeng ZS, Shia J, Weiser MR, Paty PB, et al. Oncogenic KRAS activates an embryonic stem cell-like program in human colon cancer initiation. Oncotarget. 2016; 7: 2159-74.
19. Scheffzek K, Ahmadian MR, Kabsch W, Wiesmüller L, Lautwein A, Schmitz F, et al. The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic ras mutants. Science. 1997; 277: 333-9.
20. Scheffzek K, Ahmadian MR, Wittinghofer A. GTPase-activating proteins: helping hands to complement an active site. Trends Biochem Sci. 1998; 23: 257-62.
21. The Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. Nature. 2014; 511: 543-50.
22. Tsuchida N, Murugan AK, Griceo M. Kirsten ras oncogene: significance of its discovery in human cancer research. Oncotarget. 2016; 7: 46717-33.
23. Waters AM, Der CJ. KRAS: the critical driver and therapeutic target for pancreatic cancer. Cold Spring Harb Perspect Med. 2017; 8: a031435.
24. Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature. 2012; 486: 532-6.
25. Scholl C, Frohling S, Dunn IF, Schinzel AC, Barbie DA, Kim SY, et al. Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells. Cell. 2009; 137: 821-34.
26. Luo J, Emanuele MJ, Li DN, Creighton CJ, Schlabach MR, Westbrook TF, et al. A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Nature. 2009; 137: 821-34.
27. Zhu ZH, Aref AR, Cohoon TJ, Barbie TU, Imamura Y, Yang SH, et al. Inhibition of KRAS-driven tumorigenicity by interruption of an autocrine cytokine circuit. Cancer Discov. 2014; 4: 452-65.
28. Padanad MS, Konstantinidou G, Venkateswaran N, Melegari M, Rindhe S, Mitsche M, et al. Fatty acid oxidation mediated by acyl-CoA synthetase long chain 3 is required for mutant KRAS lung
efficiently reduces tumor development in mice with K-RAS.

Liu M, Sjogren AKM, Karlsson C, Ibrahim MX, Andersson KME, Olofsson FJ, et al. Targeting the protein prenyltransferases synergistically increases genomic instability, causing synthetic lethality or tumorigenesis in a dosage-dependent manner. Cancer Res. 2010; 70: 9693-702.

Ryan MB, Corcoran RB. Therapeutic strategies to target RAS-mutant cancers. Nat Rev Clin Oncol. 2018; 15: 709-20.

Ashar HR, James L, Gray K, Carr D, McGuirk M, Maxwell E, et al. Pericyte and smooth muscle cell migration in KRAS-driven cancers. Int J Clin Oncol. 2017; 22: 651-9.

Cho KJ, van der Hoeven D, Hancock JF. Fendiline inhibits K-Ras plasma membrane localization and blocks K-Ras signal transmission. Mol Cell Biol. 2013; 33: 237-51.

Lerner EC, Qian YM, Blaskovich MA, Fossum RD, Vogt A, Sun JZ, et al. Ras CAAX peptidomimetic FTI-277 selectively blocks oncogenic Ras signaling by inducing cytoplasmic accumulation of inactive Ras-Raf complexes. J Biol Chem. 1995; 270: 26802-6.

Marciano D, Ben-Baruch G, Marom M, Egozi Y, Haklai R, Kloo Y. Farnesyl derivatives of rigid carboxylic acids-inhibitors of Ras-dependent cell growth. J Med Chem. 1995; 38: 1267-72.

Nagasu T, Yoshimatsu K, Rowell C, Lewis MD, Garcia AM. Inhibition of human tumor xenograft growth by treatment with the farnesyl transferase inhibitor B956. Cancer Res. 1995; 55: 5310-4.

Liu M, Bryant MS, Chen JP, Lee S, Yarenko B, Lipari P, et al. Antitumor activity of SCH 66336, an orally bioavailable tricyclic inhibitor of farnesyl protein transferase, in human tumor xenograft models and wap-ras transgenic mice. Cancer Res. 1998; 58: 4947-56.

Njoroge FG, Taveras AG, Kelly J, Remiszewski S, Mallams AK, Wolin R, et al. (+)-4-[2-[4-(8-Chloro-3,10-dibromo-6-11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]-pyridin-11(1H)-yl]-1-piperidinyl]-2-oxo-ethyl]-1-piperidinecarboxamide (SCH-66336): a very potent farnesyl protein transferase inhibitor as a novel antitumor agent. J Med Chem. 1998; 41: 4890-902.

Sun JZ, Qian YM, Hamilton AD, Sebti SM. Both farnesyltransferase and geranylgeranyltransferase I inhibitors are required for inhibition of oncogenic K-Ras prenylation but each alone is sufficient to suppress human tumor growth in nude mouse xenografts. Oncogene. 1998; 16: 1467-73.

Ashar HR, James L, Gray K, Carr D, McGuirk M, Maxwell E, et al. The farnesyl transferase inhibitor SCH 66336 induces a G_{1}→S or G_{1} pause in sensitive human tumor cell lines. Exp Cell Res. 2001; 262: 17-27.

Rose WC, Lee FY, Fairchild CR, Lynch M, Monticello T, Kramer RA, et al. Preclinical antitumor activity of BMS-214662, a highly apoptotic and novel farnesyltransferase inhibitor. Cancer Res.
59. Ahmadian MR, Zor T, Vogt D, Kabsch W, Selinger Z, Stubi G, et al. Guanosine triphosphatase stimulation of the farnesyl transferase inhibitor FTI-2153, blocks bipolar spindle formation and chromosome alignment and causes prometaphase accumulation during mitosis of human lung cancer cells. Neoplasia. 2000; 2: 261-72.

60. Klaus M, et al. Targeting Ras signaling through inhibition of carboxyl metabolism: an unexpected property of metothrexate. Proc Natl Acad Sci USA. 2003; 100: 6529-34.

61. Winter-Vann AM, Baron RA, Wong W, dela Cruz J, York JD, Gooden DM, et al. A small-molecule inhibitor of isoprenylcysteine carboxyl methyltransferase with antitumor activity in cancer cells. Proc Natl Acad Sci USA. 2005; 102: 4336-41.

62. Weisz B, Giehl K, Gana-Weisz M, Egozi Y, Ben-Baruch G, Marciano D, et al. A new functional Ras antagonist inhibits human pancreatic tumor growth in nude mice. Oncogene. 1999; 18: 2579-88.

63. Rivona TG, Quatela SE, Bodemann BO, Ahern IM, Soksis MJ, Mor A, et al. PKC regulates a farnesyl-electrostatic switch on KRas that promotes its association with Bcl-XL on mitochondria and induces apoptosis. Mol Cell. 2006; 21: 481-93.

64. Weisz B, Giehl K, Gana-Weisz M, Egozi Y, Ben-Baruch G, Marciano D, et al. A new functional Ras antagonist inhibits human pancreatic tumor growth in nude mice. Oncogene. 1999; 18: 2579-88.

65. Cho KJ, Park JH, Piggott AM, Salim AA, Gorfe AA, Parton RG, et al. Staurosporines disrupt phosphatidylserine trafficking and mislocalize Ras proteins. J Biol Chem. 2012; 287: 43573-84.

66. Patracci MP, Jenes MB, Li LS, Hansen R, Peters U, Kessler LV, et al. Selective inhibition of oncogenic KRAS protein with small molecules targeting the inactive state. Cancer Discov. 2016; 6: 316-29.

67. Malumbres M, Barbacid M. Ras oncogenes: the first 30 years. Nat Rev Cancer. 2003; 3: 459-65.

68. Ahmadian MR, Stege P, Scheffzek K, Wittinghofer A. Confirmation of the arginine-finger hypothesis for the GAP-stimulated GTP-hydrolysis reaction of Ras. Nat Struct Biol. 1997; 4: 686-9.

69. Ahmadian MR, Zor T, Vogt D, Kabsch W, Selinger Z, Wittinghofer A, et al. Guanosine triphosphatase stimulation of oncogenic Ras mutants. Proc Natl Acad Sci USA. 1999; 96: 7065-70.

70. Leshchiner ES, Parkhtiko A, Bird GH, Lucairelli J, Bellairs JA, Escudero S, et al. Direct inhibition of oncogenic KRAS by hydrocarbon-stapled SOS1 helices. Proc Natl Acad Sci USA. 2015; 112: 1761-6.

71. Winter JTG, Anderson M, Blades K, Brassington G, Breeze AL, Chresta C, et al. Small molecule binding sites on the Ras: SOS complex can be exploited for inhibition of Ras activation. J Med Chem. 2015; 58: 2265-74.

72. Sogabe S, Kamada Y, Miwa M, Niida A, Sameshima T, Kamaura M, et al. Crystal structure of a human K-Ras G12D mutant in complex with GDP and the cyclic inhibitory peptide KRpep-2d. ACS Med Chem Lett. 2017; 8: 732-6.

73. Durrant DE, Morrison DK. Targeting the Raf kinases in human cancer: the Raf dimer dilemma. Br J Cancer. 2018; 118: 3-8.

74. Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM, KRas(G12C) inhibitors allosterically control GTP affinity and effector interactions. Nature. 2013; 503: 548-51.

75. Athulari-Divakar SK, Vazquez-Del Carpio R, Dutta K, Baker SJ, Cosenza SC, Basi L, et al. A small molecule RAS-mimetic disrupts Ras association with effector proteins to block signaling. Cell. 2016; 165: 643-55.

76. Tavares AG, Remiszewski SW, Doll RJ, Cesar D, Huang EC, Kirschmeier P, et al. Ras oncoprotein inhibitors: the discovery of potent, ras nucleotide exchange inhibitors and the structural determination of a drug-protein complex. Bioorg Med Chem. 1997; 5: 125-33.

77. Herrmann C, Block C, Geisen C, Haas K, Weber C, Wind G, et al. Sulindac sulfide inhibits Ras signaling. Oncogene. 1998; 17: 1769-76.

78. Villalonga P, López-Alcalá C, Bosch M, Chilochees A, Rocamora N, Gil J, et al. Calmodulin binds to K-Ras, but not to H- or N-Ras, and modulates its downstream signaling. Mol Cell Biol. 2001; 21: 7345-54.

79. Palmioli A, Sacco E, Airolci D, di Nicolantonio F, D’Urzo A, Shirasawa S, et al. Selective cytotoxicity of a bicyclic Ras inhibitor in cancer cells expressing K-Ras(G12D). Biochem Biophys Res Commun. 2009; 386: 593-7.

80. Patgiri A, Yadav KK, Arora PS, Bar-Sagi D. An orthosteric inhibitor of the Ras-Sos interaction. Nat Chem Biol. 2011; 7: 585-7.

81. Maurer T, Garrenton LS, Oh A, Pitts K, Anderson DJ, Skelton NJ, et al. Small-molecule ligands bind to a distinct pocket in Ras and inhibit SOS-mediated nucleotide exchange activity. Proc Natl Acad Sci USA. 2012; 109: 5299-304.

82. Sun Q, Burke JP, Phan J, Burns MC, Olejniczak ET, Watson AG, et al. Discovery of small molecules that bind to K-Ras and inhibit SOS-mediated activation. Angew Chem. 2012; 124: 6244-7.
Hoeflich KP, Merchant M, Orr C, Chan J, den Otter D, Berry L, et al. Development of siRNA payloads to target \(-\)mutant 2017; 18: 1338.

Adamska A, Domenichini A, Falasca M. Pancreatic ductal Chem. 2015; 290: 15892-903.

expressed Ras (E-Ras), a unique Ras family member, correlates with its additional motifs and its structural properties. J Biol

Nakhaei-Rad S, Nakhaeizadeh H, Kordes C, Cirstea IC, Schmick the search continues. Future Med Chem. 2011; 3: 1787-808.

Tomasini P, Walia P, Labbe C, Jao K, Leighl NB. Targeting the KRAS pathway in non-small cell lung cancer. Oncologist. 2016; 2: 1450-60.

Baines AT, Xu DP, Der CJ. Inhibition of Ras for cancer treatment: the search continues. Med Chem. 2011; 3: 1787-808.

Nakahari-Rad S, Nakhaeizadeh H, Kordes C, Cirstea IC, Schmick M, Dvorsky R, et al. The function of embryonic stem cell-expressed Ras (E-Ras), a unique Ras family member, correlates with its additional motifs and its structural properties. J Biol Chem. 2015; 290: 15892-903.

Adamska A, Domenichini A, Falasca M. Pancreatic ductal adenocarcinoma: current and evolving therapies. Int J Mol Sci. 2017; 18: 1338.

Yuan TL, Fellmann C, Lee CS, Ritchie CD, Thapar V, Lee LC, et al. Development of siRNA payloads to target KRAS-mutant cancer. Cancer Discov. 2014; 4: 1182-97.

Hoeflich KP, Merchant M, Orr C, Chan J, den Otter D, Berry L, et al. Intermittent administration of MEK inhibitor GDC-0973 plus PI3K inhibitor GDC-0941 triggers robust apoptosis and tumor growth inhibition. Cancer Res. 2012; 72: 210-9.

Hoeflich KP, O'Brien C, Boyd Z, Cavet G, Guerrero S, Jung K, et al. In vivo antitumor activity of MEK and phosphatidylinositol 3-kinase inhibitors in basal-like breast cancer models. Clin Cancer Res. 2009; 15: 4649-64.

Holt SV, Logie A, Davies BR, Alfreder D, Runswick S, Fenton S, et al. Enhanced apoptosis and tumor growth suppression elicited by combination of MEK (Selumetinib) and mTOR kinase inhibitors (AZD8055). Cancer Res. 2012; 72: 1804-13.

Engelman JA, Chen L, Tan XH, Crosby K, Guimaraes AR, Upadhyay R, et al. Effective use of PI3K and MEK inhibitors to treat mutant \(Kras\) G12D and PIK3CA H1047R murine lung cancers. Nat Med. 2008; 14: 1351-6.

Sos ML, Fischer S, Ullrich R, Peifer M, Heuckmann JM, Koker M, et al. Identifying genotype-dependent efficacy of single and combined PI3K-and MAPK-pathway inhibition in cancer. Proc Natl Acad Sci USA. 2009; 106: 18351-6.

Wilhelm SM, Carter C, Tang LY, Wilkie D, McNabola A, Rong H, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res. 2004; 64: 7099-109.

Chen Z, Cheng K, Walton Z, Wang YC, Ehi B, Shimamura T, et al. A murine lung cancer co-clinical trial identifies genetic modifiers of therapeutic response. Nature. 2012; 483: 613-7.

Garon EB, Finn RS, Hosmer W, Dering J, Gntcher A, Adhami S, et al. Identification of common predictive markers of in vitro response to the mek inhibitor selumetinib (AZD6244; ARRY-142886) in human breast cancer and non-small cell lung cancer cell lines. Mol Cancer Ther. 2010; 9: 1955-64.

Bairros C, et al. Selumetinib plus docetaxel for KRAS-mutant advanced non-small-cell lung cancer: a randomised, multicentre, placebo-controlled, phase 2 study. Lancet Oncol. 2013; 14: 38-47.

Mas C, Boda B, CaulFuty M, Huang S, Wizniewski L, Constant S. Antitumour efficacy of the selumetinib and trametinib MEK inhibitors in a combined human airway-tumour-stroma lung cancer model. J Biotechnol. 2015; 205: 111-9.

Fernandes MS, Melo S, Velho S, Carneiro P, Carneiro F, Seruca R. Specific inhibition of p110alpha subunit of PI3K: putative therapeutic strategy for KRAS mutant colorectal cancers. Oncotarget. 2016; 7: 68346-58.

Mirzoeva OK, Das D, Heiser LM, Bhattacharya S, Siwak D, Gendelman R, et al. Basal subtype and MAPK/ERK kinase (MEK)-phosphoinositide 3-kinase feedback signaling determine susceptibility of breast cancer cells to MEK inhibition. Cancer Res. 2009; 69: 3401-6.
Cunha JEM, et al. Antisense therapy specific to mutated K-ras: Small RNA combination therapy for lung cancer. Proc Natl Acad Sci USA. 2014; 111: E3553-61.

Xue W, Dahlman JE, Tammela T, Khan OF, Sood S, Dave A, et al. Preclinical and clinical development of siRNA-based therapeutics. Adv Drug Deliv Rev. 2015; 87: 108-19.

Ozcan G, Ozpolat B, Coleman RL, Mauger M, Salito L, Barbagallo D, et al. Specific alterations of microRNA transcriptome and oncogenic pathways in colorectal cancer cells reveals a coordinate program of gene repression. Oncogene. 2013; 32: 8042.

113. Kazmi HR, Chandra A, Kumar S, Satyam HK, Gupta A, Nigam J, et al. A let-7 microRNA binding site polymorphism in the KRAS 3'UTR is associated with increased risk and reduced survival for gallbladder cancer in North Indian population. J Cancer Res Clin Oncol. 2016; 142: 2577-83.

117. Graziano F, Canestrari E, Loupakis F, Ruzzo A, Galluccio N, Santini D, et al. Genetic modulation of the Let-7 microRNA binding to KRAS 3'-untranslated region and survival of metastatic colorectal cancer patients treated with salvage cetuximab-irinotecan. Pharmacogenomics J. 2010; 10: 458-64.

119. Peng Y, Croce CM. The role of MicroRNAs in human cancer. Signal Transduct Target Ther. 2016; 1: 15004.

120. MacFarlane LA, Murphy PR. MicroRNA: biogenesis, function and role in cancer. Curr Genomics. 2010; 11: 537-61.

123. Chen X, Guo X, Zhang H, Xiang Y, Chen J, Yin Y, et al. Role of miR-143 targeting KRAS in colorectal tumorigenesis. Oncogene. 2009; 28: 1385-92.

124. Aguilar J, et al. and let-7a micro-RNA regulate K-Ras and TP53 in colorectal cancer. PLoS One. 2013; 8: e70604.
of Let-7g microRNA inhibits the proliferation and migration via K-Ras/HMGA2/snail axis in hepatocellular carcinoma. BioMed Res Int. 2014; 2014: 742417.

144. Gastaldi C, Bertetto T, Xu N, Bourget-Ponzio I, Lebrigand K, Fourre S, et al. miR-193b/365a cluster controls progression of epidermal squamous cell carcinoma. Carcinogenesis. 2014; 35: 1110-20.

145. Liao WT, Ye YP, Zhang NJ, Li TT, Wang SY, Cui YM, et al. MicroRNA-30b functions as a tumour suppressor in human colorectal cancer by targeting KRAS, PIK3CD and BCL2. J Pathol. 2014; 232: 415-27.

146. Tanaka M, Suzuki HI, Shibahara J, Kunita A, Isagawa T, Yoshimi A, et al. EV11 oncogene promotes KRAS pathway through suppression of microRNA-96 in pancreatic carcinogenesis. Oncogene. 2014; 33: 2454-63.

147. Zhang Y, Kim J, Mueller AC, Dey B, Yang Y, Lee DH, et al. Multiple receptor tyrosine kinases converge on microRNA-134 to control KRAS, STAT5B, and glioblastoma. Cell Death Differ. 2014; 21: 720-34.

148. Downward J. RAS synthetic lethal screens revisited: still seeking the elusive prize? Clin Cancer Res. 2015; 21: 1802-9.

149. Steckel M, Molina-Arcas M, Weigelt B, Marani M, Warne PH, Kuznetsov H, et al. Determination of synthetic lethal interactions in KRAS oncogene-dependent cancer cells reveals novel therapeutic targeting strategies. Cell Res. 2012; 22: 1227-45.

150. Kumar MS, Hancock DC, Molina-Arcas M, Steckel M, East P, Diefenbacher M, et al. The GATA2 transcriptional network is requisite for RAS oncogene-driven non-small cell lung cancer. Cell. 2012; 149: 642-55.

151. Corcoran RB, Cheng KA, Hata AN, Faber AC, Ebi H, Coffee EM, et al. Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. Cancer Cell. 2013; 23: 121-8.

152. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. Nature. 2009; 462: 108-12.

153. Tan N, Wong M, Nannini MA, Hong R, Lee LB, Price S, et al. Bcl-2/Bcl-X inhibition increases the efficacy of MEK inhibition alone and in combination with PI3 kinase inhibition in lung and pancreatic tumor models. Mol Cancer Ther. 2013; 12: 853-64.

154. Khosravi-Far R, Solski PA, Clark GJ, Kinch MS, Der CJ. Activation of Rac1, RhoA, and mitogen-activated protein kinases is required for Ras transformation. Mol Cell Biol. 1995; 15: 6443-53.

155. Guo W, Wu SH, Liu JS, Fang BL. Identification of a small molecule with synthetic lethality for K-ras and protein kinase C iota. Cancer Res. 2008; 68: 7403-8.

156. Vicent S, Chen R, Sayles LC, Lin CW, Walker RG, Gillespie AK, et al. Wilms tumor i (WT1) regulates KRAS-driven oncogenesis and senescence in mouse and human models. J Clin Invest. 2010; 120: 3940-52.

157. Wang Y, Ngo VN, Marani M, Yang Y, Wright G, Staudt LM, et al. Critical role for transcriptional repressor Snail2 in transformation by oncogenic RAS in colorectal carcinoma cells. Oncogene. 2010; 29: 4658-70.

158. Davidson BL, McCray Jr PB. Current prospects for RNA interference-based therapies. Nat Rev Genet. 2011; 12: 329-40.

159. Moreno PMD, Pego AP. Therapeutic antisense oligonucleotides against cancer: hurding to the clinic. Front Chem. 2014; 2: 87.

160. Gandhi AK, Shi T, Li MY, Jungnelius U, Romano A, Taberner J, et al. Immunomodulatory effects in a phase II study of lenalidomide combined with cetuximab in refractory KRAS-mutant metastatic colorectal cancer patients. PLoS One. 2013; 8: e80437.

161. Coelho MA, de Cárne Trécesson S, Rana S, Zechin D, Moore C, Molina-Arcas M, et al. Oncogenic RAS signaling promotes tumor immunoresistance by stabilizing PD-L1 mRNA. Immunity. 2017; 47: 1083-99.

162. Ebert PJR, Cheung J, Yang YG, McNamara E, Hong R, Moskalenko M, et al. MAP kinase inhibition promotes T cell and anti-tumor activity in combination with PD-L1 checkpoint blockade. Immunity. 2016; 44: 609-21.

163. Tran E, Robbins PF, Lu YC, Prickett TD, Gartner JJ, Jia L, et al. T-cell transfer therapy targeting mutant KRAS in cancer. N Engl J Med. 2016; 375: 2255-62.

164. Dong ZY, Zhong WZ, Zhang XC, Su J, Xie Z, Liu SY, et al. Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. Clin Cancer Res. 2017; 23: 3012-24.

165. Rüüvi NA, Helledman MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015; 348: 124-8.

166. Palmer DH, Dueland S, Valle JW, Aksnes AK. A phase I/II trial of TG01/GM-CSF and gemcitabine as adjuvant therapy for treating patients with resected RAS-mutant adenocarcinoma of the pancreas. J Clin Oncol. 2017; 35: 4119.

167. Mazieres J, Tolaney S, Paz-Ares L, Pujol JL, Goksel T, Lin CC, et al. P2.06-012: phase 2 study of abemaciclib+ pembrolizumab in KRAS mutation, PD-L1+, stage IV non-small cell or squamous cell lung cancer: topic: phase II+ NK. J Thorac Oncol. 2017; 12: S1076-7.

168. Valz G, Buzás EI, Szállási Z, Kalmár A, Krenács T, Tulassay Z, et al. Perspective: bidirectional exosomal transport between cancer stem cells and their fibroblast-rich microenvironment during metastasis formation. npj Breast Cancer. 2018; 4: 18.

169. Fearon ER, Wicha MS. KRAS and cancer stem cells in APC-mutant colorectal cancer. J Natl Cancer Inst. 2014; 106: dju444.

170. Najumudeen A, Jaiswal A, Lectez B, Oeteen-Lindholm C, Guzmán C, Siljamäki E, et al. Cancer stem cell drugs target K-ras signaling in a stemness context. Oncogene. 2016; 35: 5248-62.

171. Ghazvini M, Sonneveld P, Kremer A, Franken P, Sacchetti A, Atlas Y, et al. Cancer stemness in Apc- vs. Apc/KRAS-driven intestinal tumorigenesis. PLoS One. 2013; 8: e73872.

172. Moon BS, Jeong WJ, Park J, Kim TI, Min DS, Choi KY. Role of oncogenic K-Ras in cancer stem cell activation by aberrant Wnt/β-
catenin signaling. J Natl Cancer Inst. 2014; 106: djt373.

173. Quinlan MP, Quatela SE, Philips MR, Settlemier J. Activated Kras, but not Hras or Nras, may initiate tumors of endodermal origin via stem cell expansion. Mol Cell Biol. 2008; 28: 2659-74.

174. Verma RK, Yu W, Shrivastava A, Shankar S, Srivastava RK. α-Mangostin-encapsulated PLGA nanoparticles inhibit pancreatic carcinogenesis by targeting cancer stem cells in human, and transgenic (KrasG12D, and KrasG12D/p53R270H) mice. Sci Rep. 2016; 6: 32743.

175. Zhao B, Wang L, Qiu H, Zhang MS, Sun L, Peng P, et al. Mechanisms of resistance to anti-EGFR therapy in colorectal cancer. Oncotarget. 2017; 8: 3980-4000.

176. Reinmuth N, Liu WB, Fan F, Jung YD, Ahmad SA, Stoeltzing O, et al. Blockade of insulin-like growth factor I receptor function inhibits growth and angiogenesis of colon cancer. Clin Cancer Res. 2002; 8: 3259-69.

177. Rhodes D, Lipps HJ. G-quadruplexes and their regulatory roles in biology. Nucleic Acids Res. 2015; 43: 8627-37.

178. Balasubramanian S, Hurley LH, Neidle S. Targeting G-quadruplexes in gene promoters: a novel anticancer strategy? Nat Rev Drug Discov. 2011; 10: 261-75.

179. Cogoi S, Paramasivam M, Membrino A, Yokoyama KK, Xodo LE. The Kras promoter responds to MYC-associated zinc finger and poly(ADP-ribose) polymerase 1 proteins, which recognize a critical quadruplex-forming GA-element. J Biol Chem. 2010; 285: 22003-16.

180. Morgan RK, Batra H, Gaerig VC, Hockings J, Brooks TA. Identification and characterization of a new G-quadruplex forming region within the KRAS promoter as a transcriptional regulator. Biochim Biophys Acta. 2016; 1859: 235-45.

181. Carrière C, Young AL, Longnecker DS, Korc M. Acute pancreatitis markedly accelerates pancreatic cancer progression in mice expressing oncogenic Kras. Biochim Biophys Res Commun. 2009; 382: 561-5.

182. Anerile BR, O’Hayer KM, Counter CM. Oncogenic ras-induced expression of cytokines: a new target of anti-cancer therapeutics. Mol Interv. 2008; 8: 22-7.

183. Ling JH, Kang YA, Zhao YR, Xia QH, Lee DF, Chang Z, et al. KrasG12D-induced IKK2/β/NF-κB activation by IL-1α and p62 feedforward loops is required for development of pancreatic ductal adenocarcinoma. Cancer Cell. 2012; 21: 105-20.

184. Fukuda A, Wang SC, Morris JP, Favelas AE, Liou A, Kim GE, et al. Stat3 and MMP7 contribute to pancreatic ductal adenocarcinoma initiation and progression. Cancer Cell. 2011; 19: 441-55.

185. de Robertis M, Massi E, Poeta ML, Carotti S, Morini S, Cecchetelli L, et al. The AOM/DSS murine model for the study of colon carcinogenesis: from pathways to diagnosis and therapy studies. J Carcinog. 2011; 10: 9.

186. Boroughs LK, DeBerardinis RJ. Metabolic pathways promoting cancer cell survival and growth. Nat Cell Biol. 2015; 17: 351-9.

187. Kerr EM, Gaude E, Turrell FK, Frezza C, Martins GP, Mutant Kras copy number defines metabolic reprogramming and therapeutic susceptibilities. Nature. 2016; 531: 110-3.

188. Ying HQ, Kimmelstiel AC, Lyssiotis CA, Hua SJ, Chu GC, Fletcher-Sanankone E, et al. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. Cell. 2012; 149: 656-70.

189. Gouw AM, Eberlin LS, Margulis K, Sullivan DK, Toal GG, Tong L, et al. Oncogene KRAS activates fatty acid synthase, resulting in specific ERK and lipid signatures associated with lung adenocarcinoma. Proc Natl Acad Sci USA. 2017; 114: 4300-5.

190. Kamphorst JJ, Cross JR, Fan J, de Stanchina E, Mathew R, White EP, et al. Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids. Proc Natl Acad Sci USA. 2013; 110: 8882-7.

191. Wong CC, Qian Y, Li XN, Xu JY, Kang W, Tong JH, et al. SLC25A22 promotes proliferation and survival of colorectal cancer cells with KRAS mutations and xenograft tumor progression in mice via intracellular synthesis of aspartate. Gastroenterology. 2016; 151: 945-60.

192. Meyers JR, Torrence ME, Danai LV, Papagiannakopoulos T, Davidson SM, Bauer MR, et al. Tissue of origin dictates branched-chain amine acid metabolism in mutant Kras-driven cancers. Science. 2016; 353: 1161-5.

193. Guo JY, Karsli-Uzunbas G, Mathew R, Aisner SC, Kamphorst JJ, Strohecker AM, et al. Autophagy suppresses progression of K-ras-induced lung tumors to oncocytes and maintains lipid homeostasis. Genes Dev. 2013; 27: 1447-61.

194. Kohler U, Ayre BG, Goodman HM, Haseloff J. Trans-splicing ribozymes for targeted gene delivery. J Mol Biol. 1999; 285: 1935-50.

195. Kim SJ, Kim JH, Yang B, Jeong JS, Lee SW. Specific and efficient regression of cancers harboring KRAS mutation by targeted RNA replacement. Mol Ther. 2017; 25: 356-67.

196. Chiu CC, Kang YL, Yang TH, Huang CH, Fang K. Ectopic expression of herpes simplex virus-thymidine kinase gene in human non-small cell lung cancer cells conferred caspase-activated apoptosis sensitized by ganciclovir. Int J Cancer. 2002; 102: 328-33.

197. Chandec C, Mooti WJ, Oncogene-induced cellular senescence. Adv Anat Pathol. 2010; 17: 42-8.

198. DiMauro T, David G. Ras-induced senescence and its physiological relevance in cancer. Curr Cancer Drug Targets. 2010; 10: 869-76.

199. Kuhlman T, Michaloglou C, Vredeveeld LCW, Douma S, van Doorn R, Desmet CG, et al. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Cell. 2008; 133: 1019-31.

200. Courtous-Cox S, Williams SMG, Reczek EE, Johnson BW, McGillicuddy LT, Johannessen CM, et al. A negative feedback signaling network underlies oncogene-induced senescence. Cancer Cell. 2006; 10: 459-72.

201. Gibney GT, Smalley KSM. An unholy alliance: cooperation between BRAF and NFI in melanoma development and BRAF inhibitor resistance. Cancer Discov. 2013; 3: 260-3.

202. Vredeveeld LCW, Possik PA, Smit MA, Meisl K, Michaloglou C, et al. Targeting KRAS oncogene
Horlings HM, et al. Abrogation of BRAFV600E-induced senescence by PI3K pathway activation contributes to melanomagenesis. Genes Dev. 2012; 26: 1055-69.

Cicenas J, Kvederaviciute K, Meskinyte I, Meskinyte-Kausiliene E, Skeberdyte A, Cicenas J. KRAS, TP53, CDKN2A, SMAD4, BRCA1, and BRCA2 mutations in pancreatic cancer. Cancers (Basel). 2017; 9: 42.

Do K, O'Sullivan Coyne G, Chen AP. An overview of the NCI precision medicine trials-NCI MATCH and MPACT. Chin Clin Oncol. 2015; 4: 31.

Illei PB, Belchis D, Tseng LH, Nguyen D, de Marchi F, Haley L, et al. Clinical mutational profiling of 1006 lung cancers by next generation sequencing. Oncotarget. 2017; 8: 96684-96.

O’Hayer KM, Brody JR. Personalized therapy for pancreatic cancer: do we need better targets, arrows, or both? Discov Med. 2016; 21: 117-23.

Cite this article as: Saliani M, Jalal R, Ahmadian MR. From basic researches to new achievements in therapeutic strategies of KRAS-driven cancers. Cancer Biol Med. 2019; 16: 435-61. doi: 10.20892/j.issn.2095-3941.2018.0530