Chinese Medical Journal®

# Role of silent mutations in *KRAS*-mutant tumors

**Jun Lu[1,2,3,4], Chao Zhou[1], Feng Pan[1], Hongyu Liu[1], Haohua Jiang[1], Hua Zhong[1,3], Baohui Han[1,2,3]**

[1]Department of Respiratory and Critical Care Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China;
[2]Shanghai Institute of Thoracic Oncology, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China;
[3]Translational Medical Research Platform for Thoracic Oncology, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China;
[4]Department of Bio-bank, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China.

**Abstract**

Silent mutations within the *RAS* gene have garnered increasing attention for their potential roles in tumorigenesis and therapeutic strategies. Kirsten-RAS (*KRAS*) mutations, predominantly oncogenic, are pivotal drivers in various cancers. While extensive research has elucidated the molecular mechanisms and biological consequences of active *KRAS* mutations, the functional significance of silent mutations remains relatively understudied. This review synthesizes current knowledge on *KRAS* silent mutations, highlighting their impact on cancer development. Silent mutations, which do not alter protein sequences but can affect RNA stability and translational efficiency, pose intriguing questions regarding their contribution to tumor biology. Understanding these mutations is crucial for comprehensively unraveling *KRAS*-driven oncogenesis and exploring novel therapeutic avenues. Moreover, investigations into the clinical implications of silent mutations in *KRAS*-mutant tumors suggest potential diagnostic and therapeutic strategies. Despite being in early stages, research on *KRAS* silent mutations holds promise for uncovering novel insights that could inform personalized cancer treatments. In conclusion, this review underscores the evolving landscape of *KRAS* silent mutations, advocating for further exploration to bridge fundamental biology with clinical applications in oncology.

**Keywords:** *KRAS* mutations; Silent mutation; Tumor; Biological characteristics

## Introduction

*RAS* is recognized as the first tumor oncogene in humans, isolated from soft-tissue tumors.[1] As one of the most common driver genes in cancers, the *RAS* oncogene consists of Harvey-RAS (*HRAS*), Neuroblastoma-RAS (*NRAS*), and Kirsten-RAS (*KRAS*).[2] *KRAS* mutation accounts for approximately 85% of *RAS* variations in human cancers. The *KRAS* encoding gene is situated on the short arm of chromosome 12 (12p11.1-12.1), comprising six exons. And, the encoded protein of *KRAS* is a guanosine-binding protein with guanosine triphosphatase activity, GTPase KRas (KRAS), consisting of either 188 or 189 amino acids and exhibiting a molecular weight of 21.6 kDa.[3]

Usually, the activity of KRAS protein is inhibited strictly in normal cells. Nevertheless, the "inactive" state of the protein is unleashed upon specific mutations in *KRAS* encoding region, such as G12C, G12D, G12S, G12V, Q61H, etc. Subsequently, the activation of downstream pathways in KRAS ultimately leads to tumorigenesis.[4] The heterogeneous and bioactivities of *KRAS* mutations have been identified extensively.[5,6] Our attention is often directed toward non-silent mutations rather than silent mutations, due to the latter historically regarded as predominantly neutral. As investigation into KRAS function deepens, silent mutations are increasingly recognized for their significant roles in tumorigenesis.[7]

In this review, we provide a comprehensive overview of the biological functions associated with *KRAS* mutations, with particular emphasis on elucidating the role of silent mutations in *KRAS*-mutant tumors.

## *KRAS* Mutation-Mediated Tumorigenesis

### *Regulation of KRAS protein activity*

The activity of KRAS protein is regulated via switching the states between "inactive" and "active" in cells. Guanosine diphosphate (GDP)-bounded KRAS protein is in an "inactive" state, while guanosine triphosphate (GTP)-bounded protein is "active" state.[8] Due to

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000003405

Jun Lu and Chao Zhou contributed equally to this work.

**Correspondence to:** Baohui Han, Department of Respiratory and Critical Care Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China
E-Mail: xkyyhan@gmail.com; 18930858216@163.com;
Hua Zhong, Department of Respiratory and Critical Care Medicine, Shanghai Chest Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200030, China
E-Mail: eddiedong8@hotmail.com



Chinese Medical Journal 2025;138(3)
**Received:** 01-05-2024; **Online:** 10-12-2024 **Edited by:** Xiangxiang Pan

GTP-bounded KRAS protein is a closed conformation, it is easily to interact with downstream pathways [Figure 1]. Simply put, the status of KRAS protein is determined by the binding nucleotide status.

Conventionally, two key factors are considered to regulate the *KRAS* state between "inactive" and "active." One of the factors is GTP-activating protein (GAP) represented by neurofibromin-1 (NF1), which can promote

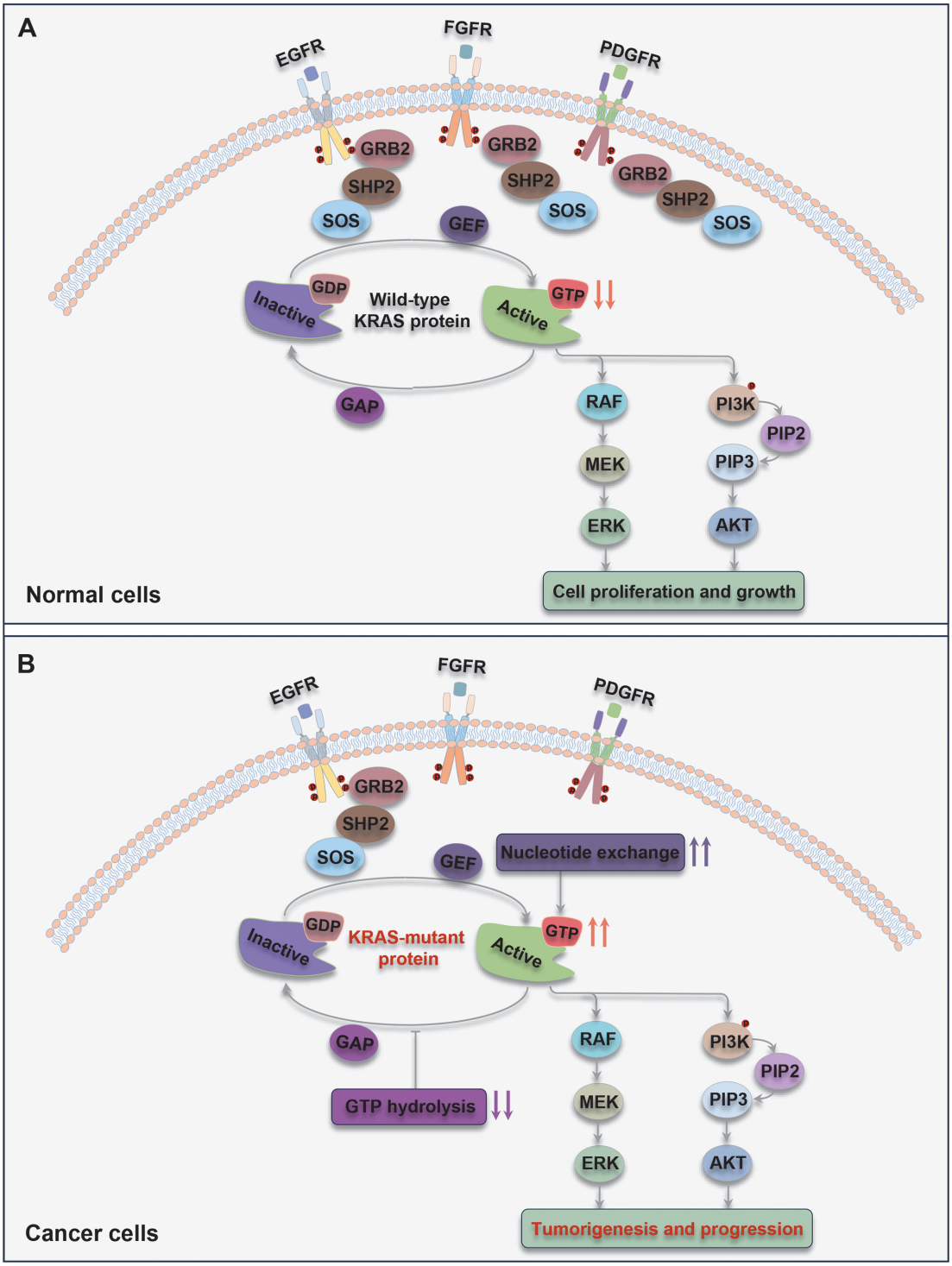


**Figure 1:** The KRAS signaling pathway functions differently in normal cells compared to cancer cells. (A) In normal cells, the activity of the KRAS protein is regulated by upstream RTKs such as EGFR, FGFR, and PDGFR. The levels of activated KRAS are determined by a balance between GTP hydrolysis and nucleotide exchange. Upon activation, KRAS initiates downstream pathways that regulate cell growth and proliferation. (B) In cancer cells, *KRAS* mutation leads to spontaneous activation of KRAS protein effects. This aberrant activation promotes cell proliferation and migration, ultimately contributing to tumorigenesis and tumor progression. AKT: Protein kinase B; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal-related kinase; FGFR: Fibroblast growth factor receptor; GAP: GTP-activating protein; GDP: Guanosine diphosphate; GEF: Guanosine exchange factor; GRB2: Growth factor receptor-bound protein 2; MEK: Mitogen-activated protein kinase/ERK kinase; GTP: Guanosine triphosphate; KRAS: Kirsten-RAS; PDGFR: Platelet-derived growth factor receptor; PI3K: Phosphoinositide 3-kinase; PIP2: Phosphatidylinositol diphosphate; PIP3: Phosphatidylinositol triphosphate; RTKs: Receptor tyrosine kinases; SHP2: Src homology 2 domain-containing protein tyrosine phosphatase; SOS: Son of sevenless.

the hydrolysis of GTP-bounded state into GDP-bounded state.[9] The other is the guanosine exchange factor (GEF) represented by the son of sevenless (SOS) protein, which catalyzes the binding of GTP to KRAS. In the normal cells without mitotic signals [Figure 1A], KRAS protein usually binds to GDP. While the normal cells transform to cancer cells due to *KRAS* mutation, the KRAS protein will binding to GTP [Figure 1B]. The state alteration is regulated through intrinsic GTP hydrolysate activity and interaction with GAP.[10]

### *KRAS*-mediated upstream and downstream pathways

The upstream signals determine the KRAS protein binds to GDP or GTP. Epidermal growth factor receptor (EGFR) has been demonstrated to mediate the downstream KRAS pathway activation.[11] Furthermore, KRAS can activate several downstream pathways, including the mitogen-activated protein kinase (MAPK) pathway and phosphoinositide 3-kinase (PI3K) pathway. Activation of these KRAS-downstream pathways contributes to maintaining tumor cell stemness [Figure 1B].

Receptor tyrosine kinases (RTKs) are recognized as important membrane protein to regulate the KRAS protein activity. RTKs such as fibroblast growth factor receptor (FGFR), platelet-derived growth factor receptor (PDGFR), EGFR, human epidermal growth factor receptor 2 (HER2), human epidermal growth factor receptor 3 (ERBB3), and human epidermal growth factor receptor 4 (ERBB4) on the cell membrane bind to corresponding ligands to phosphorylate themselves, and then activate the downstream signal proteins. When growth factor receptor-bound protein 2 (GRB2) binding to EGFR, it becomes one the best activators for KRAS pathway. Furthermore, GRB2 mediates the recruitment/activation of Src homology 2 domain-containing protein tyrosine phosphatase (SHP2), which in turn recruit SOS and activate KRAS protein.[12] Then, the activated KRAS protein-mediate downstream pathways activation can transmit the signals to the nucleus, leading to the activation of transcription factors (TFs) contribute to the cell proliferation and growth [Figure 1]. Downstream pathways of KRAS include MAPK (RAF/mitogen-activated protein kinase/ERK kinase [MEK]/extracellular signal-related kinase [ERK]) pathway[13] and PI3K (PI3K/protein kinase B [AKT]/ mammalian target of rapamycin [mTOR]) pathway.[14] In the MAPK (RAF/MEK/ERK) pathway, the GTP-binding KRAS protein promotes the RAF recruitment, resulting in the dimerization and phosphorylation of RAF. Activated RAF further mediates MEK phosphorylation, ultimately activating ERK in a highly selective manner. In the PI3K (PI3K/AKT/mTOR) pathway, the GTP-binding KRAS protein activates PI3K via phosphorylation, leading to the transformation of phosphatidylinositol diphosphate (PIP2) into phosphatidylinositol triphosphate (PIP3). Subsequently, PIP3 activates the protein kinase AKT, ultimately mediating mTOR activation.

### Activity and frequency of KRAS mutation in human cancers

In normal cells, KRAS protein activity is strictly regulated by GEF and GAP. However, *KRAS* mutations disrupt this balance. When oncogenic mutations occur in *KRAS* gene, the encoding KRAS protein disrupts the GAP-mediated hydrolysis of GTP,[4] leading to the persistent activation of KRAS and subsequent activation of downstream pathways, including MAPK, PI3K, and Ral guanine nucleotide exchange factors. Finally, the activation of these downstream pathways promotes cell proliferation and migration, ultimately contributing to tumorigenesis and progression [Figure 1B].

*KRAS* mutations are prevalent in approximately 20% of human cancers, with the highest frequency in pancreatic cancer (approximately 86%), followed by colorectal cancer (approximately 41%), and lung cancer (approximately 35% in Western population, Asian population showed lower percentage) [Figure 2A left].[5,15–17] The primary mutations in *KRAS* are frequently observed at codons 12, 13, or 61, while lower-frequency mutations usually occur at codons 63, 117, 119, and 146.[4,18] Overall, the prevalence of *KRAS* mutations at codons in common cancers was as follows: 72.2% at codon 12, 9.8% at codon 13, 14.8% at codon 61, and 3.2% at codon 146 [Figure 2A right]. However, the incidence of *KRAS* mutations fluctuates based on the origin of tumor cells and tissues.

The heterogeneity of *KRAS* subtypes underscores the diverse mutational landscapes in various cancers. For instance, Q61 mutations are prevalent in multiple myeloma (MM), G12 mutations account for nearly all cases (92%) in pancreatic adenocarcinoma (PAAD), and colorectal adenocarcinoma (COAD) exhibits a high occurrence of G13 and A146 mutations [Figure 2B]. The mutation frequency at the same locus is also diverse [Figure 2C]. For mutations at G12 locus, the predominant mutation in PAAD and COAD is $KRAS^{G12D}$. While in lung adenocarcinoma (LUAD), the most prevalent mutation is $KRAS^{G12C}$. The frequency of $KRAS^{G12R}$ in PAAD is higher at 18%, surpassing the frequencies observed in COAD at 2% and LUAD at 1%.

## Biological Properties of *KRAS* Mutation

### Wild-type KRAS allele

Wild-type *KRAS* allele exhibits growth inhibition in *KRAS* mutant tumors. The loss of the wild-type *KRAS* allele enhances mutation-induced tumorigenesis in *KRAS* mutant tumors, and the allele imbalance can impact the tumor's response to treatment.[19] Wild-type *KRAS* is believed to exert growth inhibition by competing for membrane locations and sharing activation regulators, downstream mediators, or signaling pathways.[20]

The mechanism underlying the growth inhibition of wild-type *KRAS* on tumors may involve the formation of dimer with the mutant KRAS protein. When wild-type and mutant dimers are disrupted, this growth-inhibiting effect disappears.[21] Furthermore, this growth inhibition can be overcome either by the loss of wild-type *KRAS* allele or an increase in the copy number of the *KRAS* mutant.[22]

### Classical KRAS mutation

Classical *KRAS* mutations frequently manifest at G12, G13, Q61, A146, etc. When these mutations occur in

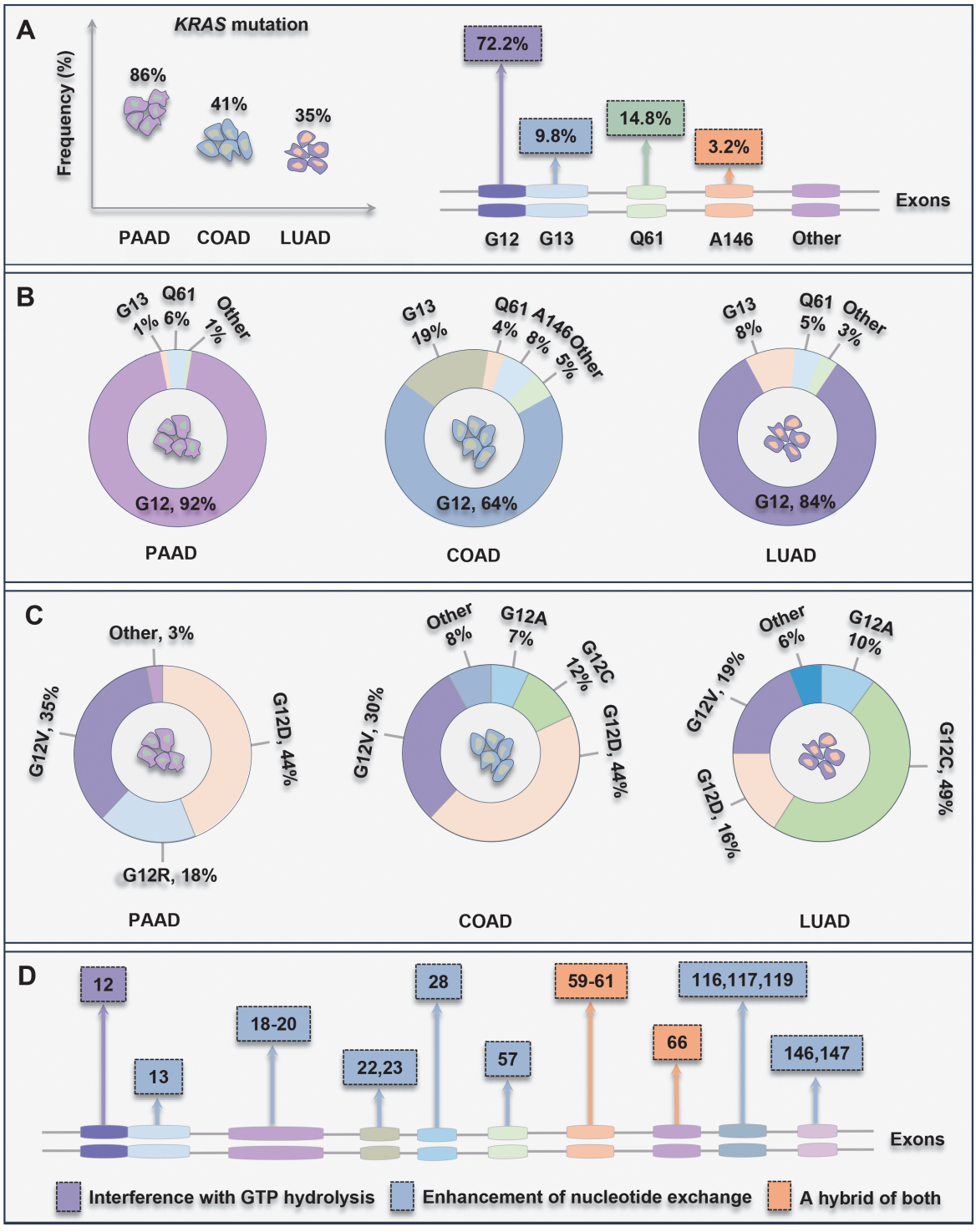


**Figure 2:** Frequency and classification of *KRAS* mutations in cancers. (A) Left: the frequency of *KRAS* mutations in PAAD, COAD, and LUAD, respectively. Right: the allele frequency of *KRAS* mutations across different exons. (B) The distribution of *KRAS* subtypes in different cancers. The majority of *KRAS* mutations in PAAD, COAD, and LUAD are found on exon 12. (C) The frequency of $KRAS^{G12}$ subtypes in PAAD, COAD, and LUAD, respectively. (D) The functional classification of *KRAS* mutations. *Source*: cBioPortal (https://www.cbioportal.org/). COAD: Colorectal adenocarcinoma; GTP: Guanosine triphosphate; *KRAS*: Kirsten-RAS; LUAD: Lung adenocarcinoma; PAAD: Pancreatic adenocarcinoma.

these loci, normal cells undergo transformation into cancer cells. However, numerous distinctions exist among various *KRAS* mutant subtypes.[10] These distinctions can be categorized into approximately four functional groups as follows: (1) interference with GTP hydrolysis; (2) enhancement of nucleotide exchange; (3) a hybrid of both; and (4) to be determined[6] [Figure 2D]. Briefly, the first category consists of G12 mutations. Any sense mutation in G12 strongly affects the hydrolytic action of GAPs on GTP, leading to elevated levels of KRAS-GTP and the activation of downstream signals.[23] The second category consists of G13, K117, A146, and other similar mutations (A18, L19, T20, Q22, L23, F28, D57, N116, D119, and K147). The primary characteristic of this category is the

strongly enhancement of nucleotide exchange, thereby strengthening KRAS's ability to bind GTP.[24] The third category consists of A59 and Q61 and other similar mutations (G60 and A66). The function of this subtype involves a combination of GTP hydrolysis and nucleotide exchange.[25] The last category does not engage in direct interactions with guanosine. The biological characteristics of KRAS proteins with these mutations need further investigation. Nevertheless, it is anticipated that such mutations may influence a wide range of KRAS functions, particularly those linked to germline mutations.[26]

When it comes to activating downstream signaling pathways, various *KRAS* mutation subtypes exhibit distinct preferences.[27] Briefly, $KRAS^{G12D}$ displays a heightened affinity for activating the PI3K/AKT signaling pathways, whereas $KRAS^{G12C}$ or $KRAS^{G12V}$ shows lower levels of phosphorylated AKT or increased RAL activation compared to other mutation subtypes.[27]

### *Clinical significance for classical KRAS mutation*

The influence of specific mutation subtypes on the biological behaviors of *KRAS* mutant tumors varies widely.[4] Distinctions have been identified between *KRAS* subtypes and clinical significances in patients with *KRAS* mutations. The prognosis and treatment response associated with *KRAS* mutations have been extensively investigated across various cancer settings.[28]

In PAAD, patients with $KRAS^{G12D}$ mutation exhibit a poorer prognosis compared to those with wild-type *KRAS*, or $KRAS^{G12R}$, or $KRAS^{G12V}$ mutations. The patients harboring $KRAS^{G12V}$ mutation, while ranking second only to $KRAS^{G12D}$, also have a worse prognosis than others.[29] Conversely, patients with the $KRAS^{G12R}$ mutation tend to fare better.[30]

In COAD, the patients with $KRAS^{G12D}$ and $KRAS^{G12V}$ mutations are associated with poorer overall survival (OS),[31,32] whereas those with $KRAS^{G13}$ mutation show the opposite trend.[31,33] In addition, patients with the $KRAS^{A146}$ mutation have better OS than those with other mutation subtypes. Regarding to the patients with the $KRAS^{Q61}$ mutation, they typically experience poorer progression-free survival (PFS) and OS.[34] According to the tumor stages, patients with limited-stage *KRAS*-mutant COAD generally exhibit a poor prognosis, whereas no significant prognostic value is associated with *KRAS* mutation in advanced COAD.[35]

In LUAD, patients with $KRAS^{G12C}$ or $KRAS^{G12V}$ mutation have a better prognosis than those with other *KRAS* mutation subtypes.[36] The favorable prognosis for patients with $KRAS^{G12C}$ or $KRAS^{G12V}$ mutations may be attributed to their better response to chemotherapy compared to other subtypes. Interestingly, these patients show a poorer response to sorafenib (a multikinase inhibitor that inhibits RAF, vascular endothelial growth factor receptor [VEGFR], etc.).[37] However, specific $KRAS^{G12C}$ inhibitors like sotorasib, adagrasib, or divarasib demonstrate excellent therapeutic efficacy and significantly prolong PFS.[38–40]

In summary, the prognosis and treatment response of *KRAS* mutations vary significantly based on factors such as subtypes, stages, and treatments. Contradictory outcomes may arise in different clinical settings, emphasizing the impact of specific *KRAS* mutation subtypes. The complexities in prognosis and treatment response are not solely attributable to KRAS function but also involve intricate interactions within upstream and downstream signaling pathways as well as genetic backgrounds.

## Silent Mutations in Tumors

Most of studies focus on non-silent mutations altering amino acid sequences in *KRAS*-mutant tumors, as they have evident biochemical effects and significant roles in tumorigenesis and tumor evolution. While the silent mutations, though less studied, are crucial components of *KRAS* mutations. Further exploration of the significance of silent mutations in *KRAS*-mutant tumors is warranted.

### *Definition of silent mutations*

The amino acid sequence of a protein is determined by codons, groups of three adjacent nucleotides, allowing for 64 possible variations. Among these, 3 are translation termination codons, and the remaining 61 encode 20 amino acids, leading to codon redundancy.[41] If a base change in a codon does not alter the encoded amino acid due to codon degeneracy, it's termed a synonymous or silent mutation.[42] While silent mutations do not affect the protein's amino acid sequence, they may impact the use of synonymous codons, introducing codon usage bias (CUB).[43] CUB, a non-random phenomenon, varies across organisms and genes within the same genome, providing insights into genetic information and preferences in translation processes.

### *Discovery of silent mutation*

The Catalogue of Somatic Mutations in Cancer (COSMIC) aims to comprehensively explore somatic mutations in human cancers, cataloging gene mutations in coding sequences.[44] Leveraging COSMIC data, numerous silent drivers of human cancers, comprising 6–8% of all driver mutations from single nucleotide substitutions, have been identified.[45] Sharma *et al*[46] analysis of 18,028 samples from 88 tumors revealed silent mutations as the second most frequent point mutation type (23.4%), surpassing nonsense mutations, deletions, and insertions. Nucleotide changes leading to silent mutations closely resemble those causing missense mutations, with non-random distribution across amino acid codes.[46] This underscores the significance of silent mutations in understanding the mutational landscape and potential functional consequences in cancer.

### *Significance of silent mutations*

Chromosomal alterations in suppressor genes and oncogenes constitute a focal point in cancer research. Historically, silent mutations, characterized by their lack of amino acid alteration, were deemed neutral and often marginalized.[7] However, advancements in high-throughput sequencing methodologies have illuminated the substantive

impact of silent mutations on the etiology, progression, and therapeutic responses of cancers [Figure 3A]. Lots of investigations underscore the non-neutral and potentially deleterious nature of silent mutations.[47] These mutations exert influence over diverse steps of protein biosynthesis, including modulating transcriptional processes, posttranscriptional regulatory mechanisms, translational efficiency, and protein stability [Figure 3B]. Therefore, inclusion of

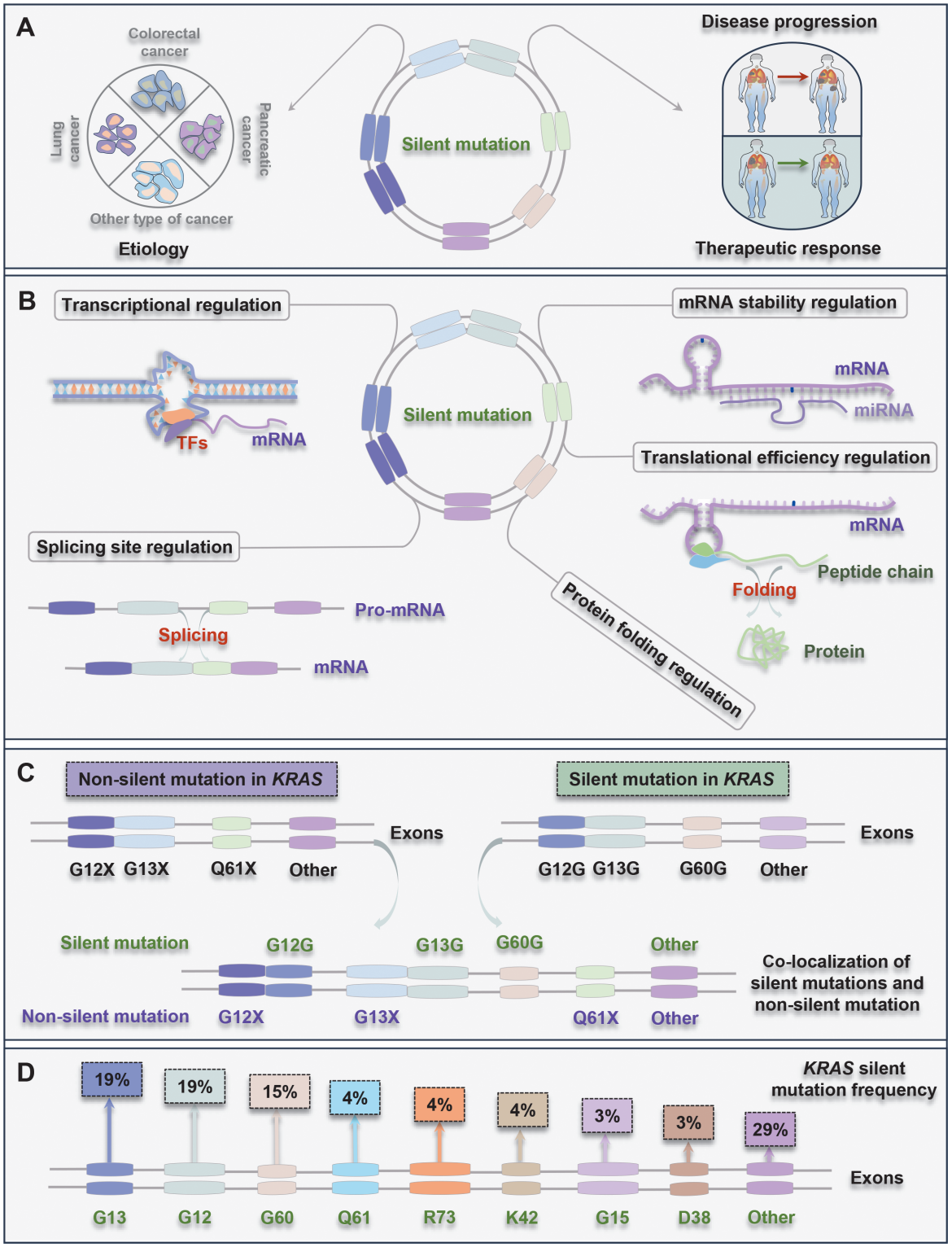


**Figure 3:** Functions of silent mutation. (A) Silent mutations play important roles in the etiology, progression, and therapeutic responses of cancers. (B) Silent mutations exert significant effects on gene expression. They regulate transcription, splicing sites, mRNA stability, translation efficiency, and protein folding. (C) Non-silent mutation and silent mutation in *KRAS*-mutant cancer. Silent mutations are often spatially adjacent to classical mutations. (D) The distribution of silent mutations in *KRAS*-mutant cancers predominantly concentrates at sites of such as G13G, G12G, and G60G. Source: COSMIC (https://cancer.sanger.ac.uk/cosmic). KRAS: Kirsten-RAS; mRNA: Messenger RNA; miRNA: MicroRNA; Pre-mRNA: Premessenger RNA; TF: Transfer factor.

silent mutations in cancer research is crucial for comprehensive understanding of the genomic landscape.

## Transcriptional and Post-transcriptional Regulation of Silent Mutation

Studies have demonstrated that silent mutations occurring in regulatory regions, such as promoters or enhancers,[48–50] can disrupt or create new TF-binding sites. TF is DNA-binding proteins interacting specifically with *cis*-acting elements in genes, play a crucial role in regulating transcription processes. The coding exon region, typically considered in the context of protein-coding, also serves as a transcriptional regulatory region. Consequently, silent mutations in this region may impact the binding of TF, potentially altering the efficiency of transcription.[51]

### *Splicing site regulation of silent mutation*

Premessenger RNA (Pre-mRNA), the initial product of transcription, undergoes critical Pre-mRNA splicing, removing intronic sequences to form mature mRNA.[52] This process, orchestrated by the spliceosome, is vital for gene expression. Splicing regulatory elements like exonic splicing enhancers (ESEs) and exonic splicing silencers (ESSs) modulate spliceosome binding, ensuring accurate splicing site identification. Disruptions in this process, caused by misidentification of exon-intron boundaries or intron retention, lead to human diseases.[53] Silent mutations, estimated to cause 15% of genetic diseases by influencing splicing regulatory sites, impact the spliceosome's composition, affinity, and function. Notably, silent mutations in *TP53*,[54] *BRCA1*,[55] *BRCA2*,[56] *APC*,[57] and *KRAS*[58] induce exon hopping, altering splicing sites and, subsequently, protein structure and function. Understanding the implications of silent mutations in splicing regulation is crucial for unraveling their role in cancer.

### *mRNA stability regulation of silent mutation*

The secondary structure of mRNA, involving stems, rings, and their combinations, is determined by the primary nucleotide sequence through base pairing.[59] Changes in individual nucleotides, such as silent mutations, hold the potential to significantly alter this secondary structure, impacting mRNA stability and consequently influencing gene expression and function.[60] A notable example is a silent mutation in *CYP2D6*, where changes in mRNA secondary structure lead to degradation and reduced mRNA expression.[61] Similarly, silent mutations in genes encoding green fluorescent protein affect mRNA degradation rates by modifying secondary structure, thereby affecting mRNA expression levels.[62] Further analysis underscores the division of codons into stable (GC3) and destabilizing (AT3) groups at the third base position, emphasizing their role in mRNA stability.[63] Micro RNA (miRNA), a regulatory RNA of approximately 22 nucleotides, plays a role in downregulating gene expression. Silent mutations can influence miRNA binding sites, inhibiting miRNA binding and subsequently increasing mRNA stability and protein expression levels.[64] In melanoma cells, silent mutations induce increased mRNA stability for the oncogene *BCL2L12*, attributed to the disruption of miR-671-5p targets within the coding sequence.[65]

### *Translational efficiency regulation of silent mutation*

Silent mutations play a pivotal role in shaping translational efficiency. These mutations exert their influence on key factors, including CUB, transfer RNA (tRNA) availability, mRNA structure, and ribosomal binding and transport. Interestingly, different codons exhibit varied translation rates, with rare codons affecting local translation control due to lower associated tRNA abundance. Silent mutations introducing rare codons diminish tRNA availability, subsequently impacting translation rates and protein function, exemplified in multidrug resistance 1 polypeptide (*MDR1*).[42] Structural alterations induced by silent mutations in mRNA modify translational efficiency, as seen in DeltaF508 of the cystic fibrosis transmembrane conductance regulator (CFTR) protein.[66] The optimal mRNAs bound more ribosomes than non-optimal ones, silent mutations can impact ribosomal binding to change translation speed.[67] Silent mutations also influence ribosomal transport, either creating pause sites that lead to translation interruptions or potentially altering the speed of peptide chains within the ribosomal tunnel.[68]

### *Protein folding regulation of silent mutation*

Silent mutations traditionally equal to synonymous changes with no impact on protein folding. However, emerging evidences challenge the viewpoint that synonymous rare codons affect translation speed and accuracy, revealing their pivotal roles in protein folding regulation, covalent modification, and expression level control.[69] The intricate interplay of codon usage, tRNA availability, ribosomal transport, and translation synthesis rate collectively influences protein folding dynamics. The conformational outcomes of cotranslational and post-translational proteins are strongly influenced by these translation dynamics, potentially leading to misfolding and altered protein structure due to modifications in translation speed.[70] Silent mutations, particularly those introducing rare codons, demonstrate the ability to significantly impact translation and cotranslational folding in proteins such as γ-B-crystallin and MDR1.[71,72] These findings suggest a previously unrecognized role for silent mutations in shaping protein folding, challenging conventional viewpoints. As silent mutations exert their influence across various stages of gene expression, including transcription, splicing, mRNA stability, and translation, their role in the intricate regulation of protein folding becomes increasingly apparent, revealing a multifaceted impact on protein concentration, structure, and function.[73]

## Silent Mutations in *KRAS*-Mutant Tumors

### *Distribution*

Usually, these silent mutations occur in close to the locations of non-silent *KRAS* mutations (G12X, G13X,

and Q61X) [Figure 3C].[4] This co-localization of silent mutations with missense mutation sites has been consistently observed in silent mutations associated with the development of cancers.[43] According to COSMIC,[44] among the 139 identified *KRAS* silent mutations, G13G (19%), G12G (19%), and G60G (15%) stand out as the most prevalent *KRAS* silent mutations [Figure 3D].

### Significance of silent mutations in KRAS-mutant tumors

In the same gene family, *KRAS* shows a higher prevalence of rare codons compared to *HRAS*. The expression of mutant KRAS proteins is constrained by these rare codons.[74] Mutations that convert the rare codons in *KRAS* to common codons have been identified to increase the expression levels of mRNA and protein.[75] Here, we highlight some recent studies in the field and those that affect KRAS expression and biological function [Table 1]. The codon bias observed in KRAS influences various aspects of the gene expression process and post-translational modifications, fostering enhanced transcription and translation efficiency.[76] Furthermore, silent mutations in *KRAS* can induce alterations in the protein structure through the process of cotranslational protein folding.[76] Codon bias also plays a key role in *KRAS*-driven resistance, providing a rationale insight for potential overcoming resistance.[77] Studies have shown that when silent mutations occur in exon 3, the translation of *KRAS* mRNA becomes more efficient, leading to an increased expression of the KRAS-mutant protein.[74,78]

The silent mutation can also induce non-silent mutations. For instance, the G60G silent mutation in *KRAS* gene eliminated the splicing regulatory site, leading to the generation of functional KRAS (Q61K) variants.[58,79] By establishing NIH3T3 cell lines with common silent *KRAS* mutations (G12G, G13G, G60G), it was observed that all *KRAS*-mutant cell lines harboring silent mutations showed heightened expression of KRAS mRNA and protein. Moreover, these *KRAS*-mutated cell lines demonstrated accelerated growth rates and increased invasiveness.[80] Therefore, this evidence suggests that silent mutations found in *KRAS* may contribute to tumorigenesis by increasing the expression levels of *KRAS* mRNA and modifying the structure of KRAS protein.

However, silent mutations in *KRAS* exhibit heterogeneous. It has been confirmed that silent mutations exert a

**Table 1: KRAS silent mutations reported in previous studies.**

| KRAS silent mutations | Variants | Codon change | Cell lines | Influence | References |
|---|---|---|---|---|---|
| G10G | c.30A>C | GGA>GGC | HEK293 | Affect the transcript secondary structure leading to KRAS protein significant increase | [46] |
| G12G | c.36 T>C | GGT>GGC | HEK293, HeLa | Strongly induce *KRAS* mRNA and protein expression | [46] |
| G12G | c.36 T>G | GGT>GGG | HEK293 | Significantly decrease KRAS protein expression | [46] |
| G13G | c.39 C>G | GGC>GGG | HEK293 | Decreases protein expression | [46] |
| G13G | c.39C>A | GGC>GGA | HEK293 | Increases mRNA and protein expression | [46] |
| 33 synonymous mutations in exon 3 | 33 synonymous mutations in exon 3 | 33 synonymous mutations in exon 3 | Mouse embryonic fibroblasts | Increased the average amount of KRAS protein | [74] |
| 33 synonymous mutations in exon 3 | 33 synonymous mutations in exon 3 | 33 synonymous mutations in exon 3 | BM KSL cells and HSCs | Increased Kras protein and Erk1/2-mediated augmentation of Cdk4/6 activation | [78] |
| G60G | c.180T>A, C, or G | GGT>GGA, GGC, GGG | PC-9 | Eliminates the splice donor site and yields a functional KRAS (Q61K) variant | [58] |
| G12G, G13G, G60G | c.36 T>G, A, Cc.39 C>G, A, Tc.180 T>G, A, C | | NIH3T3 | Expressed much more KRAS protein<br>Cause increases in proliferation and saturation density<br>More invasive in multiple assays | [80] |

HSCs: Hematopoietic stem cell; KRAS: Kirsten-RAS; mRNA: Messenger RNA.

notable impact on KRAS expression in the context of *KRAS* G12G mutations. Specifically, the c.36 T>C (G12G) variant strongly enhances the expression of KRAS mRNA and protein, while the c.36 T>G (G12G) variant has the opposite effect, significantly reducing the expression of KRAS mRNA and protein.[46] Interestingly, in different tumor cell lines with the same *KRAS* silent mutation, the biological function of silent mutations is not exactly the same.[76] In HepG2 cells, the effects of codon usage on KRAS protein and RNA were much smaller than those in the other cell lines. In contrast, in Huh7 cells the differences were larger than those seen in HEK-293T cells.[76] This variability might be attributed to differences in tRNA concentrations across distinct tumor cell lines, impacting the translational efficiency of KRAS.[81] Moreover, these distinctions could be interconnected with intricate signaling pathways or diverse genetic backgrounds.

### Study prospectives of silent mutations in KRAS-mutant tumors

The landscape of cancer research has historically been dominated by the exploration of non-silent mutations. However, recent advancements in the understanding of silent mutations, particularly in the context of *KRAS*, have opened up new prospectives for investigation and potential therapeutic interventions. New databases containing information on silent mutations, such as Pan-Cancer Analysis of Whole Genome (PCAWG)[82] and Synonymous Mutations in Cancer Database (SynMICdb),[46] also attach importance to silent mutations. Undergoing a paradigm shift, these databases have now recognized the substantial significance of silent mutations. This transformation provides researchers with access to valuable insights into the intricate world of silent mutations within the context of *KRAS* mutant tumors.

The development of gene editing technologies has empowered researchers to understand the functions of the specific *KRAS* silent mutation. Using the clustered regularly interspaced short palindromic repeats (CRISPR) editing makes the modification and study of the *KRAS* gene more accessible.[83] Designing a method to generate different alleles of *KRAS* mutations in cell lines has become more straightforward. By editing tumors with specific *KRAS* silent mutations, researchers can comprehensively investigate the distinct biological traits associated with these mutations.[58]

### Targeting specific KRAS silent mutations for clinical use

The promising strategy of therapeutic targeting of *KRAS* silent mutations should be considered for incorporation into future clinical practice. Targeting *KRAS* silent mutations through therapeutic approaches like antisense oligonucleotides (ASOs) represents a promising strategy in clinical oncology.[84] ASOs, due to their ability to target mRNA with high precision and minimal side effects, have emerged as a viable option.[85] In recent years, the exploration of ASO-based therapies has manifested in 100 phase I trials, with 25% progressing to phase II/III trials.[84]

Targeting *KRAS* mutations with ASO could be a promising strategy for clinical use. For example, AZD4785, a high-affinity constrained ethyl-containing therapeutic ASO targeting *KRAS* mRNA, reduces *KRAS* gene expression.[86,87] In a groundbreaking study by Kobayashi *et al*[58], intratumoral injection of oligonucleotides demonstrated a reduction in target lesions in the patients with $KRAS^{G60G}$ silent mutations. They designed mutation-specific oligonucleotides to target ESE motif-mediated splicing, rendering the KRAS (Q61) protein non-functional. This innovative therapeutic strategy provides new possibilities for precision medicine, suggesting that targeting specific *KRAS* silent mutations could be a viable approach in treating of *KRAS* mutant cancers.

## Conclusions

*KRAS* mutations have been extensively studied in recent decades, and *KRAS* mutations lead to the activation of KRAS protein, which in turn continuously activates downstream pathways contributing to tumorigenesis and progression. The biological characteristics of different *KRAS* mutation subtypes are discussed in this review. Here, we aim to deepen our understanding of the biological traits of silent mutations within *KRAS*-mutant tumors through an extensive literature review. Research on *KRAS* silent mutations is just beginning in the exploration of *KRAS* functions and clinical practices. This indicates that there is still much that we have yet to uncover, presenting ample opportunities for discovering novel insights and implementing them in clinical practice.

### Funding

This work was supported by grants from the National Natural Science Foundation of China (Nos. 82473102, 82272913, 82301980, and 82072573), National Multi-disciplinary Treatment Project for Major Disease (No. 2020NMDTP), Chinese Society of Clinical Oncology (Nos. Y-2019AZZD-0355 and Y-QL2019-012), and Shanghai Jiao Tong University (No. YG2021QN12).

### Conflicts of interest

None.

## References

1. Harvey JJ. An unidentified virus which causes the rapid production of tumours in mice. Nature 1964;204:1104–1105. doi: 10.1038/2041104b0.
2. Hobbs GA, Der CJ, Rossman KL. RAS isoforms and mutations in cancer at a glance. J Cell Sci 2016;129:1287–1292. doi: 10.1242/jcs.182873.
3. Lowy DR, Willumsen BM. Function and regulation of ras. Annu Rev Biochem 1993;62:851–891. doi: 10.1146/annurev.bi.62.070193.004223.
4. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: Weaving a tumorigenic web. Nat Rev Cancer 2011;11:761–774. doi: 10.1038/nrc3106.
5. Cook JH, Melloni GEM, Gulhan DC, Park PJ, Haigis KM. The origins and genetic interactions of KRAS mutations are allele- and tissue-specific. Nat Commun 2021;12:1808. doi: 10.1038/s41467-021-22125-z.

6. Johnson C, Burkhart DL, Haigis KM. Classification of KRAS-activating mutations and the implications for therapeutic intervention. Cancer Discov 2022;12:913–923. doi: 10.1158/2159-8290.Cd-22-0035.
7. Sauna ZE, Kimchi-Sarfaty C. Understanding the contribution of synonymous mutations to human disease. Nat Rev Genet 2011;12:683–691. doi: 10.1038/nrg3051.
8. Kranenburg O. The KRAS oncogene: Past, present, and future. Biochim Biophys Acta 2005;1756:81–82. doi: 10.1016/j.bbcan.2005.10.001.
9. Xu GF, O'Connell P, Viskochil D, Cawthon R, Robertson M, Culver M, *et al*. The neurofibromatosis type 1 gene encodes a protein related to GAP. Cell 1990;62:599–608. doi: 10.1016/0092-8674(90)90024-9.
10. Simanshu DK, Nissley DV, McCormick F. RAS proteins and their regulators in human disease. Cell 2017;170:17–33. doi: 10.1016/j.cell.2017.06.009.
11. Liebmann C. Regulation of MAP kinase activity by peptide receptor signalling pathway: Paradigms of multiplicity. Cell Signal 2001;13:777–785. doi: 10.1016/s0898-6568(01)00192-9.
12. Lin C-C, Wieteska L, Suen KM, Kalverda AP, Ahmed Z, Ladbury JE. Grb2 binding induces phosphorylation-independent activation of Shp2. Commun Biol 2021;4:437. doi: 10.1038/s42003-021-01969-7.
13. Braicu C, Buse M, Busuioc C, Drula R, Gulei D, Raduly L, *et al*. A comprehensive review on MAPK: A promising therapeutic target in cancer. Cancers (Basel) 2019;11:1618. doi: 10.3390/cancers11101618.
14. Vanhaesebroeck B, Perry MWD, Brown JR, Andre F, Okkenhaug K. PI3K inhibitors are finally coming of age. Nat Rev Drug Discov 2021;20:741–769. doi: 10.1038/s41573-021-00209-1.
15. Prior IA, Hood FE, Hartley JL. The frequency of ras mutations in cancer. Cancer Res 2020;80:2969–2974. doi: 10.1158/0008-5472.Can-19-3682.
16. Li X, Liu H, Huang B, Yang M, Fan J, Zhang J, *et al*. Schistosoma infection, KRAS mutation status, and prognosis of colorectal cancer. Chin Med J2024;137:235–237. doi: 10.1097/CM9.0000000000002905.
17. Wang C, Shao J, Song L, Ren P, Liu D, Li W. Persistent increase and improved survival of stage I lung cancer based on a large-scale real-world sample of 26,226 cases. Chin Med J 2023;136:1937–1948. doi: 10.1097/CM9.0000000000002729.
18. Zehir A, Benayed R, Shah RH, Syed A, Middha S, Kim HR, *et al*. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med 2017;23:703–713. doi: 10.1038/nm.4333.
19. Junttila MR, Karnezis AN, Garcia D, Madriles F, Kortlever RM, Rostker F, *et al*. Selective activation of p53-mediated tumour suppression in high-grade tumours. Nature 2010;468:567–571. doi: 10.1038/nature09526.
20. Young A, Lou D, McCormick F. Oncogenic and wild-type ras play divergent roles in the regulation of mitogen-activated protein kinase signaling. Cancer Discov 2013;3:112–123. doi: 10.1158/2159-8290.CD-12-0231.
21. Ambrogio C, Köhler J, Zhou ZW, Wang H, Paranal R, Li J, *et al*. KRAS dimerization impacts MEK inhibitor sensitivity and oncogenic activity of mutant KRAS. Cell 2018;172:857–868.e15. doi: 10.1016/j.cell.2017.12.020.
22. Burgess MR, Hwang E, Mroue R, Bielski CM, Wandler AM, Huang BJ, *et al*. KRAS allelic imbalance enhances fitness and modulates MAP kinase dependence in cancer. Cell 2017;168:817–829. doi: 10.1016/j.cell.2017.01.020.
23. Hunter JC, Manandhar A, Carrasco MA, Gurbani D, Gondi S, Westover KD. Biochemical and structural analysis of common cancer-associated KRAS mutations. Mol Cancer Res 2015;13:1325–1335. doi: 10.1158/1541-7786.MCR-15-0203.
24. Johnson CW, Lin YJ, Reid D, Parker J, Pavlopoulos S, Dischinger P, *et al*. Isoform-specific destabilization of the active site reveals a molecular mechanism of intrinsic activation of KRas G13D. Cell Rep 2019;28:1538–1550.e7. doi: 10.1016/j.celrep.2019.07.026.
25. Zhou ZW, Ambrogio C, Bera AK, Li Q, Li XX, Li L, *et al*. KRAS(Q61H) preferentially signals through MAPK in a RAF dimer-dependent manner in non-small cell lung cancer. Cancer Res 2020;80:3719–3731. doi: 10.1158/0008-5472.CAN-20-0448.
26. Schubbert S, Bollag G, Lyubynska N, Nguyen H, Kratz CP, Zenker M, *et al*. Biochemical and functional characterization of germ line KRAS mutations. Mol Cell Biol 2007;27:7765–7770. doi: 10.1128/MCB.00965-07.
27. Munoz-Maldonado C, Zimmer Y, Medova M. A comparative analysis of individual RAS mutations in cancer biology. Front Oncol 2019;9:1088. doi: 10.3389/fonc.2019.01088.
28. Haigis KM. KRAS alleles: The devil is in the detail. Trends Cancer 2017;3:686–697. doi: 10.1016/j.trecan.2017.08.006.
29. Cheng H, Liu C, Jiang J, Luo G, Lu Y, Jin K, *et al*. Analysis of ctDNA to predict prognosis and monitor treatment responses in metastatic pancreatic cancer patients. Int J Cancer 2017;140:2344–2350. doi: 10.1002/ijc.30650.
30. Qian ZR, Rubinson DA, Nowak JA, Morales-Oyarvide V, Dunne RF, Kozak MM, *et al*. Association of alterations in main driver genes with outcomes of patients with resected pancreatic ductal adenocarcinoma. JAMA Oncol 2018;4:e173420. doi: 10.1001/jamaoncol.2017.3420.
31. Imamura Y, Morikawa T, Liao X, Lochhead P, Kuchiba A, Yamauchi M, *et al*. Specific mutations in KRAS codons 12 and 13, and patient prognosis in 1075 BRAF wild-type colorectal cancers. Clin Cancer Res 2012;18:4753–4763. doi: 10.1158/1078-0432.CCR-11-3210.
32. Jones RP, Sutton PA, Evans JP, Clifford R, McAvoy A, Lewis J, *et al*. Specific mutations in KRAS codon 12 are associated with worse overall survival in patients with advanced and recurrent colorectal cancer. Br J Cancer 2017;116:923–929. doi: 10.1038/bjc.2017.37.
33. Margonis GA, Kim Y, Spolverato G, Ejaz A, Gupta R, Cosgrove D, *et al*. Association between specific mutations in KRAS codon 12 and colorectal liver metastasis. JAMA Surg 2015;150:722–729. doi: 10.1001/jamasurg.2015.0313.
34. Taieb J, Balogoun R, Le Malicot K, Tabernero J, Mini E, Folprecht G, *et al*. Adjuvant FOLFOX plus/- cetuximab in full RAS and BRAF wildtype stage III colon cancer patients. Ann Oncol 2017;28:824–830. doi: 10.1093/annonc/mdw687.
35. Roth AD, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, *et al*. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: Results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol 2010;28:466–474. doi: 10.1200/JCO.2009.23.3452.
36. Izar B, Zhou H, Heist RS, Azzoli CG, Muzikansky A, Scribner EE, *et al*. The prognostic impact of KRAS, its codon and amino acid specific mutations, on survival in resected stage I lung adenocarcinoma. J Thorac Oncol 2014;9:1363–1369. doi: 10.1097/JTO.0000000000000266.
37. Ihle NT, Byers LA, Kim ES, Saintigny P, Lee JJ, Blumenschein GR, *et al*. Effect of KRAS oncogene substitutions on protein behavior: Implications for signaling and clinical outcome. J Natl Cancer Inst 2012;104:228–239. doi: 10.1093/jnci/djr523.
38. Hong DS, Fakih MG, Strickler JH, Desai J, Durm GA, Shapiro GI, *et al*. KRAS(G12C) inhibition with sotorasib in advanced solid tumors. N Engl J Med 2020;383:1207–1217. doi: 10.1056/NEJMoa1917239.
39. Jänne PA, Riely GJ, Gadgeel SM, Heist RS, Ou SI, Pacheco JM, *et al*. Adagrasib in non–small-cell lung cancer harboring a KRASG12C mutation. N Engl J Med 2022;387:120–131. doi: 10.1056/NEJMoa2204619.
40. Sacher A, LoRusso P, Patel MR, Miller WH Jr., Garralda E, Forster MD, *et al*. Single-agent divarasib (GDC-6036) in solid tumors with a KRAS G12C mutation. N Engl J Med 2023;389:710–721. doi: 10.1056/NEJMoa2303810.
41. Subramaniam AR, Pan T, Cluzel P. Environmental perturbations lift the degeneracy of the genetic code to regulate protein levels in bacteria. Proc Natl Acad Sci U S A 2013;110:2419–2424. doi: 10.1073/pnas.1211077110.
42. Plotkin JB, Kudla G. Synonymous but not the same: The causes and consequences of codon bias. Nat Rev Genet 2011;12:32–42. doi: 10.1038/nrg2899.
43. Hunt RC, Simhadri VL, Iandoli M, Sauna ZE, Kimchi-Sarfaty C. Exposing synonymous mutations. Trends Genet 2014;30:308–321. doi: 10.1016/j.tig.2014.04.006.
44. Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N, *et al*. COSMIC: The catalogue of somatic mutations in cancer. Nucleic Acids Res 2019;47:D941–D947. doi: 10.1093/nar/gky1015.
45. Supek F, Minana B, Valcarcel J, Gabaldon T, Lehner B. Synonymous mutations frequently act as driver mutations in human cancers. Cell 2014;156:1324–1335. doi: 10.1016/j.cell.2014.01.051.
46. Sharma Y, Miladi M, Dukare S, Boulay K, Caudron-Herger M, Groß M, *et al*. A pan-cancer analysis of synonymous mutations. Nat Commun 2019;10:2569. doi: 10.1038/s41467-019-10489-2.

47. Shen X, Song S, Li C, Zhang J. Synonymous mutations in representative yeast genes are mostly strongly non-neutral. Nature 2022;606:725–731. doi: 10.1038/s41586-022-04823-w.
48. Zhou S, Hawley JR, Soares F, Grillo G, Teng M, Madani Tonekaboni SA, *et al*. Noncoding mutations target cis-regulatory elements of the FOXA1 plexus in prostate cancer. Nat Commun 2020;11:441. doi: 10.1038/s41467-020-14318-9.
49. Melton C, Reuter JA, Spacek DV, Snyder M. Recurrent somatic mutations in regulatory regions of human cancer genomes. Nat Genet 2015;47:710–716. doi: 10.1038/ng.3332.
50. Liu Y, Yang Q, Zhao F. Synonymous but not silent: The codon usage code for gene expression and protein folding. Annu Rev Biochem2021;90:375–401.doi:10.1146/annurev-biochem-071320-112701.
51. Stergachis AB, Haugen E, Shafer A, Fu W, Vernot B, Reynolds A, *et al*. Exonic transcription factor binding directs codon choice and affects protein evolution. Science 2013;342:1367–1372. doi: 10.1126/science.1243490.
52. Martinez NM, Gilbert WV. Pre-mRNA modifications and their role in nuclear processing. Quant Biol 2018;6:210–227. doi: 10.1007/s40484-018-0147-4.
53. Faustino NA, Cooper TA. Pre-mRNA splicing and human disease. Genes Dev 2003;17:419–437. doi: 10.1101/gad.1048803.
54. Cartegni L, Chew SL, Krainer AR. Listening to silence and understanding nonsense: Exonic mutations that affect splicing. Nat Rev Genet 2002;3:285–298. doi: 10.1038/nrg775.
55. Raponi M, Kralovicova J, Copson E, Divina P, Eccles D, Johnson P, *et al*. Prediction of single-nucleotide substitutions that result in exon skipping: Identification of a splicing silencer in BRCA1 exon 6. Hum Mutat 2011;32:436–444. doi: 10.1002/humu.21458.
56. Hansen TVO, Steffensen AY, Jonson L, Andersen MK, Ejlertsen B, Nielsen FC. The silent mutation nucleotide 744 G ->A, Lys-172Lys, in exon 6 of BRCA2 results in exon skipping. Breast Cancer Res Treat 2010;119:547–550. doi: 10.1007/s10549-009-0359-4.
57. Montera M, Piaggio F, Marchese C, Gismondi V, Stella A, Resta N, *et al*. A silent mutation in exon 14 of the APC gene is associated with exon skipping in a FAP family. J Med Genet 2001;38:863–867. doi: 10.1136/jmg.38.12.863.
58. Kobayashi Y, Chhoeu C, Li J, Price KS, Kiedrowski LA, Hutchins JL, *et al*. Silent mutations reveal therapeutic vulnerability in RAS Q61 cancers. Nature 2022;603:335–342. doi: 10.1038/s41586-022-04451-4.
59. Mortimer SA, Kidwell MA, Doudna JA. Insights into RNA structure and function from genome-wide studies. Nat Rev Genet 2014;15:469–479. doi: 10.1038/nrg3681.
60. Wu Q, Medina SG, Kushawah G, DeVore ML, Castellano LA, Hand JM, *et al*. Translation affects mRNA stability in a codon-dependent manner in human cells. Elife 2019;8:e45396. doi: 10.7554/eLife.45396.
61. Toscano C, Raimundo S, Klein K, Eichelbaum M, Schwab M, Zanger UM. Silent mutation (2939G >A, exon 6; CYP2D6*59) leading to impaired expression and function of CYP2D6. Pharmacogenet Genomics 2006;16:767–770. doi: 10.1097/01.fpc.0000236331.03681.24.
62. Chen S, Li K, Cao W, Wang J, Zhao T, Huan Q, *et al*. Codon-resolution analysis reveals a direct and context-dependent impact of individual synonymous mutations on mRNA level. Mol Biol Evol 2017;34:2944–2958. doi: 10.1093/molbev/msx229.
63. Hia F, Yang SF, Shichino Y, Yoshinaga M, Murakawa Y, Vandenbon A, *et al*. Codon bias confers stability to human mRNAs. EMBO Rep 2019;20:e48220. doi: 10.15252/embr.201948220.
64. Bartel DP. MicroRNAs: Target recognition and regulatory functions. Cell 2009;136:215–233. doi: 10.1016/j.cell.2009.01.002.
65. Gartner JJ, Parker SC, Prickett TD, Dutton-Regester K, Stitzel ML, Lin JC, *et al*. Whole-genome sequencing identifies a recurrent functional synonymous mutation in melanoma. PNAS 2013;110:13481–13486. doi: 10.1073/pnas.1304227110.
66. Bartoszewski RA, Jablonsky M, Bartoszewska S, Stevenson L, Dai Q, Kappes J, *et al*. A synonymous single nucleotide polymorphism in delta F508 CFTR alters the secondary structure of the mRNA and the expression of the mutant protein. J Biol Chem 2010;285:28741–28748. doi: 10.1074/jbc.M110.154575.
67. Heyer Erin E, Moore Melissa J. Redefining the translational status of 80S monosomes. Cell 2016;164:757–769. doi: 10.1016/j.cell.2016.01.003.
68. Barrington CL, Galindo G, Koch AL, Horton ER, Morrison EJ, Tisa S, *et al*. Synonymous codon usage regulates translation initiation. Cell Rep 2023;42:113413. doi: 10.1016/j.celrep.2023.113413.
69. Faure G, Ogurtsov AY, Shabalina SA, Koonin EV. Adaptation of mRNA structure to control protein folding. RNA Biol 2017;14:1649–1654. doi: 10.1080/15476286.2017.1349047.
70. Bartoszewski R, Króliczewski J, Piotrowski A, Jasiecka AJ, Bartoszewska S, Vecchio-Pagan B, *et al*. Codon bias and the folding dynamics of the cystic fibrosis transmembrane conductance regulator. Cell Mol Biol Lett 2016;21:23. doi: 10.1186/s11658-016-0025-x.
71. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, *et al*. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 2007;315:525–528. doi: 10.1126/science.1135308.
72. Buhr F, Jha S, Thommen M, Mittelstaet J, Kutz F, Schwalbe H, *et al*. Synonymous codons direct cotranslational folding toward different protein conformations. Mol Cell 2016;61:341–351. doi: 10.1016/j.molcel.2016.01.008.
73. Hia F, Takeuchi O. The effects of codon bias and optimality on mRNA and protein regulation. Cell Mol Life Sci 2021;78:1909–1928. doi: 10.1007/s00018-020-03685-7.
74. Pershing NLK, Lampson BL, Belsky JA, Kaltenbrun E, MacAlpine DM, Counter CM. Rare codons capacitate Kras-driven de novo tumorigenesis. J Clin Invest 2015;125:222–233. doi: 10.1172/JCI77627.
75. Lampson BL, Pershing NL, Prinz JA, Lacsina JR, Marzluff WF, Nicchitta CV, *et al*. Rare codons regulate KRas oncogenesis. Curr Biol 2013;23:70–75. doi: 10.1016/j.cub.2012.11.031.
76. Fu J, Dang Y, Counter C, Liu Y. Codon usage regulates human KRAS expression at both transcriptional and translational levels. J Biol Chem 2018;293:17929–17940. doi: 10.1074/jbc.RA118.004908.
77. Ali M, Kaltenbrun E, Anderson GR, Stephens SJ, Arena S, Bardelli A, *et al*. Codon bias imposes a targetable limitation on KRAS-driven therapeutic resistance. Nat Commun 2017;8:15617. doi: 10.1038/ncomms15617.
78. Sasine JP, Himburg HA, Termini CM, Roos M, Tran E, Zhao L, *et al*. Wild-type Kras expands and exhausts hematopoietic stem cells. JCI Insight 2018;3:e98197. doi: 10.1172/jci.insight.98197.
79. Molina-Arcas M, Downward J. The potency of a KRAS silent variant. N Engl J Med 2022;386:2523–2525. doi: 10.1056/NEJMcibr2202981.
80. Waters AM, Bagni R, Portugal F, Hartley JL. Single synonymous mutations in KRAS cause transformed phenotypes in NIH3T3 cells. PLoS One 2016;11:e0163272. doi: 10.1371/journal.pone.0163272.
81. Dittmar KA, Goodenbour JM, Pan T. Tissue-specific differences in human transfer RNA expression. PLoS Genet 2006;2:2107–2115. doi: 10.1371/journal.pgen.0020221.
82. ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. Nature 2020;578:82–93. doi: 10.1038/s41586-020-1969-6.
83. Katti A, Diaz BJ, Caragine CM, Sanjana NE, Dow LE. CRISPR in cancer biology and therapy. Nat Rev Cancer 2022;22:259–279. doi: 10.1038/s41568-022-00441-w.
84. Crooke ST, Baker BF, Crooke RM, Liang XH. Antisense technology: An overview and prospectus. Nat Rev Drug Discov 2021;20:427–453. doi: 10.1038/s41573-021-00162-z.
85. Rodgers G, Austin C, Anderson J, Pawlyk A, Colvis C, Margolis R, *et al*. Glimmers in illuminating the druggable genome. Nat Rev Drug Discov 2018;17:301–302. doi: 10.1038/nrd.2017.252.
86. Linnane E, Davey P, Zhang P, Puri S, Edbrooke M, Chiarparin E, *et al*. Differential uptake, kinetics and mechanisms of intracellular trafficking of next-generation antisense oligonucleotides across human cancer cell lines. Nucleic Acids Res 2019;47:4375–4392. doi: 10.1093/nar/gkz214.
87. Katsuzaki Y, Tsukimura R, Chandela A, Chano T, Ueno Y. 4′-C-aminoethoxy-modified DNAs exhibit increased nuclease resistance, sustained RNase H activity, and inhibition of KRAS gene expression. Chem Biodivers 2022;19:e202200125. doi: 10.1002/cbdv.202200125.


**How to cite this article:** Lu J, Zhou C, Pan F, Liu HY, Jiang HH, Zhong H, Han BH. Role of silent mutations in *KRAS*-mutant tumors. Chin Med J 2025;138:278–288. doi: 10.1097/CM9.0000000000003405