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Post-translational modifications of KRAS: from molecular regulation to therapeutic targeting

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Highlights:

- PTMs regulate RAS membrane localization, trafficking, and spatial signalling organization.
- PTMs modulate RAS nucleotide cycling, effector engagement, protein stability, and degradation.
- Targeting RAS PTM networks offers new therapeutic opportunities for RAS-driven cancers.

Abstract: The rat sarcoma virus (RAS) proteins are small GTPases that regulate cell signaling and are frequently mutated in human cancers. Their activity is mainly controlled by cycling between guanosine diphosphate (GDP)-bound and guanosine triphosphate (GTP)-bound states, which is regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). In addition to this classical mechanism, post-translational modifications (PTMs) provide another important layer of RAS regulation. RAS proteins undergo several types of modification, including farnesylation, proteolysis, methylation, palmitoylation, phosphorylation, ubiquitylation, nitrosylation, ADP-ribosylation, and glucosylation. These modifications can influence RAS localization, stability, activity, and interactions with other signaling proteins. As a result, post-translational modifications can influence the strength, duration and location of RAS signaling, and thereby contribute to tumor development, progression and treatment response. The recent success of direct RAS inhibitors has overturned the long-standing perception of RAS as an undruggable target and has renewed interest in the broader regulatory network that controls RAS function. Enzymes that install, remove or recognize RAS modifications may provide complementary therapeutic opportunities and potential strategies to overcome resistance. In this Review, we discuss the molecular mechanisms and functional consequences of RAS post-translational modifications,

their interplay with oncogenic mutations, and emerging therapeutic approaches targeting RAS and its modification-dependent regulatory machinery.

Keywords: RAS; post-translational modifications (PTMs); GTPase; guanine nucleotide exchange factors (GEFs); GTPase-activating proteins (GAPs); inhibitors

1. Introduction

Rat sarcoma virus (RAS) mutations are widely prevalent in human malignancies, with an overall mutation frequency of approximately 19%–30% [1]. Large-scale genomic studies have shown that KRAS is the RAS subtype with the highest mutation frequency. KRAS mutations are particularly common in pancreatic ductal adenocarcinoma, colorectal cancer (CRC), and lung adenocarcinoma [2,3]. NRAS mutations are more commonly found in melanoma and certain hematological malignancies. The overall frequency of HRAS mutations is relatively low, but they have a clear carcinogenic effect in head and neck squamous cell carcinoma and bladder cancer [4,5]. The hotspot mutations of RAS are mainly most commonly at codons 12, 13, and 61. RAS mutations impair the GTPase activity and lead to abnormal activation of downstream signaling pathways such as MAPK/ERK and PI3K/AKT [6].

RAS proteins are a class of small GTPases that regulates cellular signaling networks involes cell proliferation, differentiation, and survival. Ras biological function depends on the precise sensing of the guanine nucleotide binding state between inactive guanosine diphosphate (GDP)-bound conformation and active guanosine triphosphate (GTP)-states. The cycling of RAS is tightly controlled by guanine nucleotide exchange factors (GEFs, such as SOS1) promote the release of GDP and the loading of GTP onto RAS to the active GTP-bound form and GTPase-activating proteins (GAPs, including NF1 and p120-RasGAP) catalyze the hydrolysis of the γ-phosphate of GTP bound to RAS return to the inactive GDP-bound state [7]. Notably, oncogenic mutations in RAS often alter its intrinsic nucleotide exchange rate or impair GAP-mediated GTP hydrolysis, leading to persistent activation and driving tumorigenesis [8] (Figure 1).

The intrinsic features of RAS present significant changes for “undruggable” target [9,10]. Because of the picomolar affinity of RAS for GTP [11–13], and absence of deep small molecule binding pockets [14,15]. Beyond mutational status and activation regulate by GEFs and GAPs, RAS proteins are further controlled by diverse post-translational modifications (PTMs) that influence localization, effector interactions, protein stability. Understanding the molecular mechanisms of RAS PTMs provides insight into the RAS signaling and but also identifies novel therapeutic targets. In this review, we systematically summarize the major types of PTMs of RAS proteins, their molecular mechanisms and biological functions, and their potential applications in targeted cancer therapy.

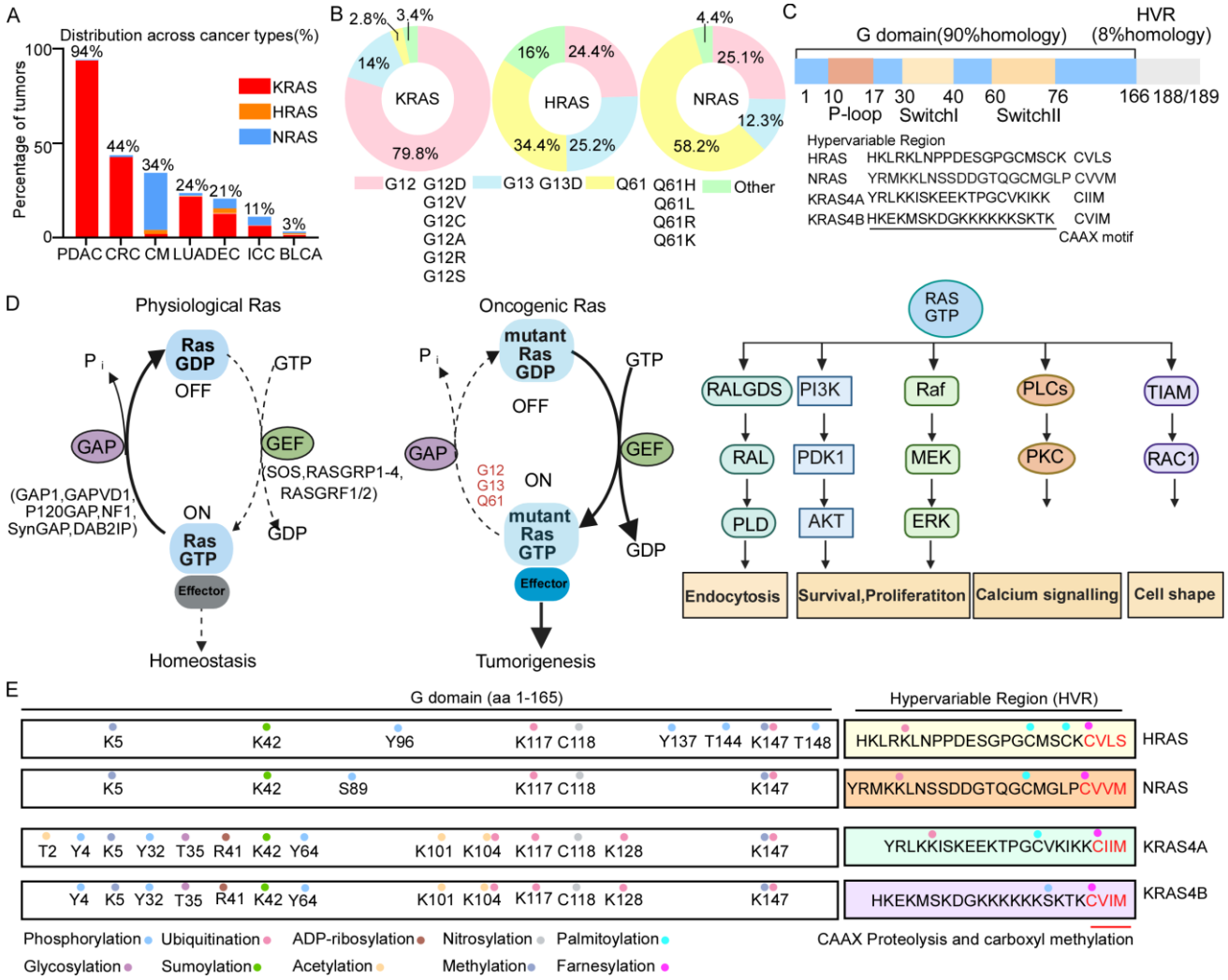

Figure 1. Overview of RAS mutation frequency and hotspots, structure, signaling, and post-translational modifications (PTMs). **(A)** Frequency of KRAS, HRAS, and NRAS mutations across various cancers. **(B)** The distribution of RAS mutation hotspots. **(C)** Structure organization of RAS, including the conserved G domain (P-loop, Switch I/II) and the hypervariable region (HVR) with the CAAX motif contribute to RAS binding to cell membrane. **(D)** RAS activation cycle by GEF/GAP-regulated GDP-GTP switching in normal *versus* mutant cells, that controls multiple signalling pathways including RAF-MEK-ERK, PI3K-AKT, RAL, PLC, and RAC pathways. **(E)** The PTM landscape of RAS isoforms. All schematic figures in this review were created with BioRender.

2. Structural and functional basis of KRAS signaling

2.1. Domain architecture and isoform-specific features of KRAS

There are three RAS genes in mammalian genomes: HRAS, NRAS, and KRAS. Because the transcript from the KRAS locus can be alternatively spliced, the three genes give rise to four protein isoforms: HRAS, NRAS, KRAS4A and KRAS4B. RAS is composed of two major structural regions: (1) the G-domain (aa 1-166) and (2) the C-terminal (aa 166-188/189) unstructured hypervariable region. The G-domain, responsible for binding GTP, plays a central role in activating downstream signaling pathways. It includes crucial structural elements, such as the phosphate-binding loop (P-loop) (aa 10-17),

Switch I (aa 30-40), and Switch II regions (aa 60-76). A conserved nucleotide-binding pocket adjacent to the Switch regions is for GTP binding. The P-loop stabilizes the phosphate groups of the bound nucleotides, while the Switch regions form critical interfaces for interacting with effector proteins and regulatory molecules, including GAPs and GEFs. HVR is the area with the most significant differences between different RAS subtypes. Although the overall sequence identity of the different RAS subtypes exceeds 85%, the homology of the HVR region is only about 30%-40%. This region mainly determines the subcellular localization and functional characteristics of different RAS subtypes. The carboxyl terminus of HVR contains the CAAX motif (C represents cysteine, A represents aliphatic amino acids, and X represents any amino acid). This motif can initiate a series of lipid-related modification processes, including isoprenylation, proteolytic cleavage, and carboxymethylation.

The vast majority of oncogenic lesions cluster at codons 12, 13 and 61 (G12, G13 and Q61), where single missense substitutions impair intrinsic and GAP-stimulated GTP hydrolysis or alter nucleotide affinity, thereby generating constitutively active KRAS oncoproteins. The impact of hotspot mutations on RAS nucleotide cycling shows significantly differences. Hunter *et al.* reported that the G13D mutation can markedly accelerated nucleotide exchange. Compared to wild-type KRAS, the GDP and GTP exchange rates of the G13D mutant are approximately 14 times and 9 times higher, respectively. Most other hotspot mutants have nucleotide exchange rates that are relatively close to those of wild-type KRAS. The endogenous GTP hydrolysis activity of wild-type KRAS is relatively weak. Different oncogenic mutations have varying effects on this process. The G12C mutant still maintains a GTP hydrolysis rate similar to that of wild-type KRAS. In contrast, mutations such as G12A, G12R, and Q61H, Q61L significantly reduce KRAS's GTPase activity, decreasing it by approximately 40-80 times. G12D, G12V, and G13D mutations cause moderate GTP hydrolysis defects.

2.2. Molecular switch function and oncogenic activation of KRAS

RAS can activate multiple signaling pathways that promote cellular malignant transformation. These pathways include the MAPK pathway, the PI3K-AKT-mTOR pathway, as well as pathways mediated by small GTPases such as Rho, Rac, Ral, and phospholipase C.

RAF-MEK-ERK is a canonical signaling pathway downstream of RAS signaling [16]. Activated KRAS-GTP recruits rapidly accelerating fibrosarcoma (RAF) kinases to the plasma membrane. RAF binds to the membrane undergoes a conformational change that promotes activation by homodimers or heterodimers. The activated RAF binds to and phosphorylates MEK1/2, MEK1/2 activates ERK1/2 through phosphorylation. Activated ERK regulates transcription and translation by phosphorylating various cytoplasmic and nuclear substrates. These substrates include ribosomal S6 kinase, ETS family transcription factors, and cell cycle regulatory proteins, thus promotes cell proliferation, differentiation, and cell fate. PI3K-AKT-mTOR is another major signaling pathway regulated by KRAS, playing a key role in growth factor supply, nutritional status, and cellular stress signals [17]. Activated KRAS directly binds to the p110 catalytic subunit of class I PI3K, converts phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3) [18]. The accumulation of PIP3 promotes the phosphoinositide-dependent protein kinase 1 (PDK1) phosphorylates AKT at Thr308 site, the AKT also phosphorylates at Ser473 site by the mTOR complex 2 (mTORC2). Activated AKT regulates cellular functions through multiple pathways. For example, AKT can activate the mTOR complex 1 (mTORC1) to promote protein synthesis, nutrient uptake, and anabolic metabolism. AKT also inhibits apoptosis by

phosphorylating Bcl-XL/Bcl-2-associated death promoters (BADs). The phosphorylation BAD binds to 14-3-3 proteins instead interact with the BCL-2, thereby promoting cell survival. The RalGDS–RAL pathway is another important downstream pathway of KRAS, in which RalGDS promotes GDP-to-GTP exchange on RAL GTPases [19]. Activated RAL proteins regulate cytoskeletal remodeling and migration via RAC and Rac/cell division cycle 42(CDC42), innate immune signaling through TANK-binding kinase 1 (TBK1), and vesicle trafficking and endocytosis through phospholipase D (PLD) activation. KRAS further modulates actin dynamics and membrane organization by TIAM1, thereby activating RAC1-dependent programs that control cell shape, adhesion and directional migration. In addition, KRAS can activate phospholipase C-ε (PLCε), thereby promoting phosphoinositide hydrolysis and calcium-dependent signaling pathways.

3. PTMs regulating membrane localization and trafficking

In addition to conformational changes and regulatory factors, the subcellular localization of Ras protein is also crucial for its function. Ras proteins are regulated by post-translational modification (PTM) such as farnesylation, palmitoylation, and methylation to be stably anchored inside the cell membrane (Figure 2) [20]. RAS is accurately localozed on the cell membrane can efficiently receive upstream signals from membrane receptors (such as RTK) and activate downstream effectors inside the membrane.

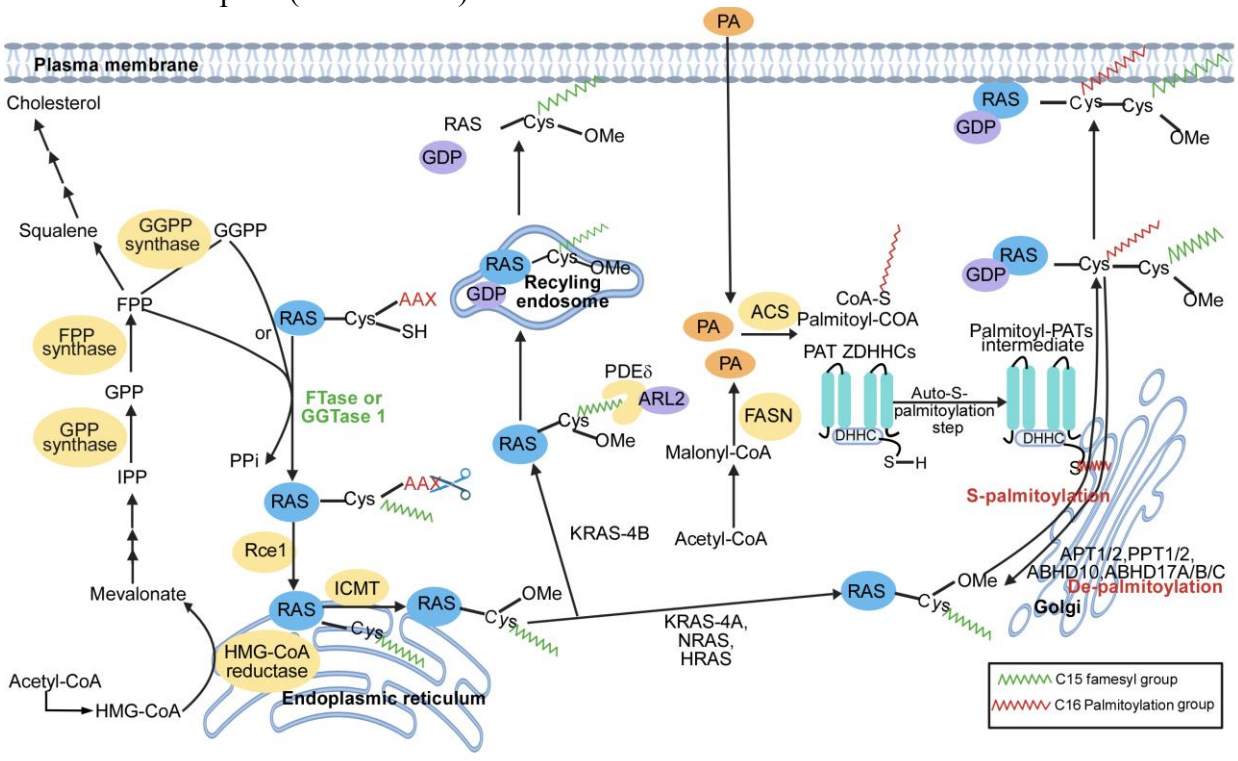

Figure 2. Post-translational lipid modifications regulate RAS membrane localization and trafficking. Farnesylation at the C-terminal CAAX motif is catalyzed by farnesyltransferase (FTase), followed by proteolytic cleavage by Rce1 and methylation by isoprenylcysteine carboxyl methyltransferase (ICMT). HRAS, NRAS, and KRAS 4A undergo reversible S-palmitoylation mediated by ZDHHC palmitoyltransferases facilitates their trafficking from the Golgi apparatus to the plasma membrane. Depalmitoylation by acyl-protein thioesterases (APT/PPT) promotes recycling of RAS through endosomal compartments. KRAS 4B primarily relies on electrostatic interactions and distinct trafficking pathways. All schematic figures in this review were created with BioRender.

3.1. CAAX motif mediated processing and maturation of RAS proteins

Farnesylation of the CAAX cysteine by farnesyltransferase (FTase) is the initiating and rate-limiting step in RAS maturation. FTase transfers a C15 isoprenoid (farnesyl group) from farnesyl pyrophosphate to the CAAX cysteine through a stable thioether linkage. This lipid modification promotes the association of newly synthesized RAS with the endoplasmic reticulum (ER), where subsequent CAAX processing occurs. Following farnesylation, RCE1 proteolytically removes the -AAX tripeptide, after which ICMT methylates the exposed prenylated cysteine, reducing the polarity of the C terminus and increasing membrane affinity [21]. The farnesyl-binding protein PDE6δ also contributes to RAS trafficking and can influence efficient RAS signalling.Because farnesylation is required for RAS membrane association and maturation, FTase became an early target for anti-RAS drug development, leading to the generation of numerous farnesyltransferase inhibitors (FTIs). However, when FTase is inhibited, KRas-4B and NRas can undergo alternative prenylation by geranylgeranyl transferase 1 (GGTase1), which installs a C20 geranylgeranyl group and can preserve membrane association. The farnesyltransferase inhibitor tipifarnib showed high potency and oral bioavailability, inhibited its intended target in vivo, and suppressed HRAS-driven tumors, but FTIs did not achieve broad clinical efficacy as anticancer agents [22–25]. ICMT represents another shared step in the post-prenylation processing of multiple RAS isoforms and has therefore also been investigated as a potential anti-RAS target. However, no ICMT inhibitors have entered clinical trials [26].

Farnesylation of KRAS at C185 increases its hydrophobicity and has been reported to promote liquid-liquid phase separation (LLPS), contributing to the formation of cytoplasmic KRAS condensates. These condensates promote clustering of ER-localized RCE1 and interact with KRAS, thereby facilitating KRAS processing and trafficking to the plasma membrane. Other hydrophobic lipid modifications, including geranylgeranylation, myristoylation, and palmitoylation, can similarly promote protein condensation. Thus, lipid modifications can influence not only membrane targeting but also protein phase behaviour, providing an additional mechanism for the spatial organization of RAS signalling [27].

3.2. Palmitoylation and dynamic membrane traffick ing

Palmitoylation is a reversible lipid modification of HRAS, NRAS, and KRAS4A that occurs in addition to constitutive farnesylation. Catalysed by DHHC-domain-containing palmitoyltransferases (PATs), this reaction transfers a C16 saturated palmitoyl group from palmitoyl-CoA to cysteine residues within the HVR through a labile thioester bond. This reversible modification supports the membrane-trafficking cycle of palmitoylated RAS isoforms. Newly synthesised RAS is palmitoylated at the Golgi and subsequently transported to the plasma membrane, where it can engage in signalling. Depalmitoylation then promotes release from the plasma membrane and redistribution toward intracellular membranes, allowing RAS to return to the Golgi for re-palmitoylation. Although APT1 and APT2 (encoded by LYPLA1 and LYPLA2) were initially implicated in this process, more recent studies have identified additional depalmitoylases with isoform-specific roles. The palmitoylation-depalmitoylation cycle is largely independent of the nucleotide-bound state of RAS but influences its membrane residence time and subcellular distribution, thereby contributing to compartment-specific signalling [28,29].

The ZDHHC family provides the principal enzymatic machinery for RAS palmitoylation. Among its members, the DHHC9-GCP16 complex is a major palmitoyltransferase for HRAS and NRAS. Structural studies indicate that GCP16 functions primarily as a stabilising and substrate-regulatory cofactor for DHHC9 rather than as a catalytic component. The contribution of individual regulators can also vary with cellular context. For example, ZDHHC18-mediated HRAS palmitoylation has been implicated in renal fibrosis, extending the relevance of this modification beyond oncogenic signalling. RAB27B has also been reported to regulate NRAS palmitoylation-dependent trafficking by controlling access to DHHC9, with stronger effects on mutant than on wild-type RAS signalling [30,31]. On the depalmitoylation side of the cycle, members of the ABHD17 family have been identified as major regulators of NRAS palmitate turnover, challenging the earlier emphasis on APT1 and APT2 [32]. Pharmacological inhibition of ABHD17 increases NRAS membrane association and has been reported to suppress NRAS-driven tumour growth in preclinical models, suggesting that altered depalmitoylation may represent a potential therapeutic vulnerability in NRAS-mutant malignancies [33].

3.3. Phosphorylation of the KRAS4B hypervariable region

Phosphorylation provides a reversible means of regulating the spatial organization and signaling output of KRAS. In KRAS4B, PKC-mediated phosphorylation of Ser181 within the hypervariable region partially neutralizes the positive charge of the polybasic domain and weakens its electrostatic interaction with anionic phospholipids. This modification does not eliminate membrane binding but reduces membrane affinity in a manner influenced by lipid composition and membrane fluidity. As a result, KRAS can redistribute from the plasma membrane to intracellular membranes, including the ER and Golgi, while retaining the capacity to form nanoclusters [34,35]. Changes in KRAS localization appear to affect signaling primarily through spatial reorganization rather than through major alterations in KRAS–RAF binding affinity, which remains largely unchanged [36]. The functional consequences of Ser181 phosphorylation are context dependent. In some settings, redistribution of KRAS disrupts ER-mitochondrial calcium signaling, impairs mitochondrial respiration, and reduces cell survival [34,37]. In epithelial tumor models, including colorectal cancer, Ser181 phosphorylation has instead been associated with maintenance of epithelial organization and polarity as well as tumor growth. Consistent with this, the nonphosphorylatable S181A mutant reduces tumor formation, whereas the phosphomimetic S181D mutant enhances MAPK and PI3K signaling and promotes tumor growth. Phosphorylated KRAS has also been detected in human tumors, supporting the physiological relevance of this modification and indicating that Ser181 phosphorylation can modulate KRAS signaling in a context-dependent manner rather than acting solely as an activating or inhibitory event [38,39].

The reversibility of KRAS phosphorylation is also supported by the identification of PTPN2 as a KRAS phosphatase. By removing inhibitory tyrosine phosphorylation, PTPN2 has been reported to maintain KRAS plasma membrane localization and downstream signaling, thereby supporting the survival of KRAS-mutant tumor cells [40].

3.4. Pathogen-mediated and endogenous ADP-ribosylation of Ras

ADP-ribosylation is a reversible post-translational modification in which ADP-ribose units derived from NAD^+ are covalently attached to specific amino acid residues on target proteins. Catalyzed by

ADP-ribosyltransferases (ARTDs/PARPs), this modification can alter protein function, localization, and signaling under both physiological and stress conditions [41–43]. Ras ADP-ribosylation (ADPr) can be broadly divided into pathogen-mediated exogenous modification and endogenous stress-induced modification in mammalian cells. Pathogen-mediated Ras ADP-ribosylation was initially characterized in studies of bacterial virulence. The opportunistic pathogen Pseudomonas aeruginosa secretes the type III effector Exoenzyme S (ExoS), which directly ADP-ribosylates Ras, with Arg41 identified as a classical modification site. Modification at this site disrupts the interaction of Ras with downstream effectors and consequently attenuates Ras-ERK signaling [44]. Beyond its direct effects on Ras signaling, ExoS-mediated Ras ADP-ribosylation suppresses Ras-dependent functions in neutrophils and other innate immune cells, supporting its contribution to P. aeruginosa-mediated evasion of host immune responses [45]. Ras proteins can also undergo endogenous ADP-ribosylation in mammalian cells, particularly during oxidative stress. Chemical biology and proteomic studies have identified Cys181 and Cys184 of H-Ras as ADP-ribosylation sites. These residues lie within the C-terminal hypervariable region and are also sites of S-palmitoylation required for proper membrane localization. ADP-ribosylation at Cys181 and Cys184 is antagonistic to palmitoylation at the same residues. Under oxidative stress, increased Ras ADP-ribosylation impairs membrane association and reduces Ras-ERK signaling output, thereby limiting excessive proliferative signaling. These observations have led to the proposal that stress-induced Ras ADP-ribosylation functions as a self-limiting mechanism that restrains Ras signaling under adverse cellular conditions [46].

4. PTMs regulating nucleotide cycling and effector engagement

Beyond membrane localization, several PTMs directly modulate the biochemical activity of RAS at the level of nucleotide exchange, GTP hydrolysis, and effector interaction. These modifications operate within or near the Switch regions and nucleotide-binding pocket, and their characterization requires particular methodological rigor given the use of modification-mimetic substitutions in many studies (Figure 3).

4.1. Phosphorylation at switch region residues

Phosphorylation has emerged as a particularly versatile mechanism that modulates RAS function by reshaping its spatial organization, conformational dynamics, and engagement with regulatory and effector proteins. Rather than acting as a binary on-off switch, phosphorylation encodes context-dependent oncogenic information that is superimposed upon the canonical GDP/GTP cycle. Reversible tyrosine phosphorylation provides an additional regulatory layer modulating KRAS signaling independently of oncogenic mutations. Src family kinases can phosphorylate Tyr32 of GTP-bound KRAS. This modification reduces the affinity of KRAS for RAF and promotes its interaction with negative regulatory factor [47]. In contrast, SHP2-mediated dephosphorylation can restore the binding of KRAS to effector proteins and reactivate the MAPK signaling pathway [48]. Structurally, Tyr32 phosphorylation increases the flexibility of the switch I region and affects nucleotide coordination [49]. Phosphorylation of Tyr64 can stabilize a different switch II conformation, thereby weakening the binding of KRAS to effector proteins. Enhanced sampling simulations further indicate that phosphorylation at these two sites can shift the conformational distribution of KRAS toward a non-classical state and alter the Mg^{2+}/GTP interaction [50–52]. RAS can also be subject to feedback regulation mediated by effector

proteins. RIN1 can recruit ABL kinase to promote the phosphorylation of RAS at the Tyr137 site. Tyr137 is located at an allosteric regulatory hotspot. Phosphorylation at this site can enhance the binding of RAS to effector proteins while weakening the conformational coupling that favors GTP hydrolysis [53]. In addition to kinase-driven feedback regulation, RAS itself can also undergo phosphorylation events with significant functional impacts. For example, the tumor-associated mutant KRAS A59T has autophosphorylation capability. This modification, on one hand, reduces GTP hydrolysis, and on the other hand, promotes nucleotide exchange [54]. Phosphorylation regulation is not limited to KRAS; it also exists in other RAS subtypes. STK19 can phosphorylate the Ser89 site of NRAS and enhance the activity of the MAPK and PI3K signaling pathways. This process can promote NRAS Q61R-driven cell transformation and also suggests that Ser89 may be a potential therapeutic target in NRAS mutant tumors [55–57].

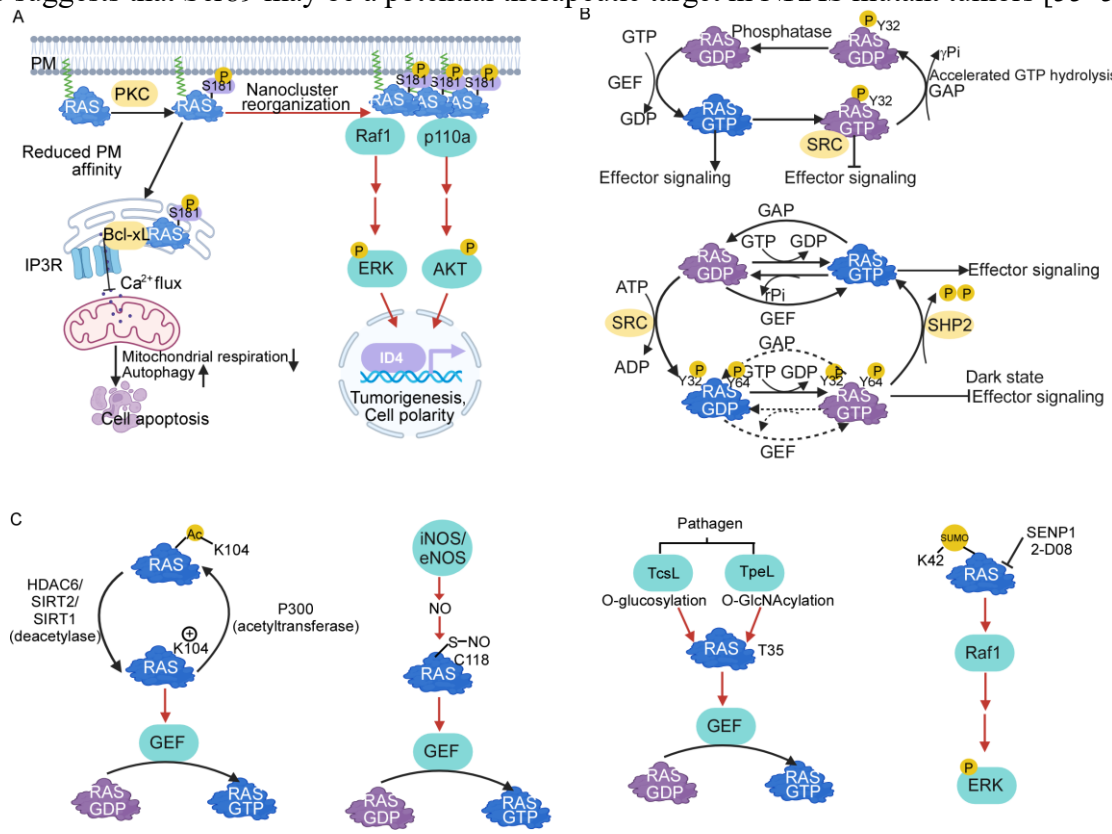

Figure 3. Phosphorylation and other non-lipid PTMs regulating RAS signaling activity. **(A)** Ser181 phosphorylation regulates KRAS membrane dynamics, signaling output and mitochondrial function; **(B)** Tyrosine phosphorylation dynamically controls the RAS activation cycle; **(C)** Additional post-translational modifications fine-tune RAS activity. Abbreviations: PM, plasma membrane; PKC, protein kinase C; GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein; SHP2, Src homology region 2-containing protein tyrosine phosphatase 2; NO, nitric oxide; iNOS, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; IP3R, inositol 1,4,5-trisphosphate receptor; SUMO, small ubiquitin-like modifier.

4.2. Site-specific monoubiquitination fine-tunes RAS signaling

In addition to protein degradation, site-specific monoubiquitination can also regulate RAS signaling in various ways, and this effect is independent of the mutational state of RAS. Some ubiquitination events can directly enhance RAS activity. For example, monoubiquitination at the K147 site can reduce RAS's

sensitivity to GAP and make its binding to RAF no longer strictly dependent on the GTP state. Ubiquitination at the K117 site can accelerate nucleotide exchange, thereby promoting the formation of the GTP-bound state of RAS [58–60]. In contrast, the ubiquitination at the K104 site primarily affects the structural state of RAS rather than directly altering its catalytic activity. In vitro biochemical experiments show that monoubiquitination at K104 has a limited impact on RAS activity. However, structural analysis and molecular simulations suggest that K104-Ub can alter the conformational distribution of RAS and influence its interaction interface with regulatory proteins through steric hindrance [61,62]. Other ubiquitination sites can inhibit RAS signaling. Ubiquitination at the K128 site can enhance the binding of RAS to GAP proteins such as NF1 and RASA1, thereby limiting signal intensity and selectively inhibiting certain downstream pathways. In KRAS-mutant pancreatic cancer models, this effect is particularly manifested as the inhibition of the RALB-TBK1 signaling axis [63]. Moreover, the ubiquitination of RAS also exhibits significant subtype differences. Multiple lysine sites on HRAS can undergo ubiquitination, and there is a certain degree of redundancy between different sites. This suggests that the ubiquitination of HRAS does not completely depend on a specific site. In contrast, the influence of the membrane environment on the accessibility of lysine sites may more significantly determine its ubiquitination-mediated membrane transport and signal output [64].

4.3. Acetylation controls KRAS conformational dynamics and signaling output

Acetylation represents another layer of post-translational regulation of RAS, although its functional consequences remain incompletely defined. In KRAS, K104 is located in loop 7 adjacent to the α2 and α3 helices and has been proposed as a regulatory site. Initial studies suggested that K104 acetylation weakens SOS-mediated nucleotide exchange and suppresses KRAS-driven cell transformation, resulting in reduced KRAS signaling activity. HDAC6 and SIRT2 were subsequently proposed as KRAS deacetylases that support KRAS signaling by maintaining K104 in a deacetylated state [65]. Consistent with this model, perturbation of K104 can impair RAF engagement and restrict invasive and proliferative phenotypes, although these effects appear to depend on the mutational and signaling context [66]. The functional consequences of K104 acetylation, however, remain debated. Studies using genetically encoded acetyl-lysine incorporation showed that authentic K104 acetylation does not fully reproduce the effects of the commonly used acetylation-mimetic mutant K104Q, complicating the interpretation of earlier experiments. Biochemical analyses further indicated that K104 acetylation produces only subtle local conformational changes while impairing both GEF-mediated nucleotide exchange and GAP-catalysed GTP hydrolysis; these opposing effects may preserve overall KRAS activation in cells [67–69]. Other studies nevertheless support regulation of oncogenic signalling through reversible K104 acetylation. P300 has been proposed to function as the acetyltransferase, whereas SIRT1 promotes KRAS signalling through K104 deacetylation within a KRAS-MYC positive-feedback loop. Pharmacological inhibition of SIRT1 has also been reported to enhance the efficacy of targeted therapy and chemotherapy [70]. RAS acetylation is not restricted to Lys104. In addition to Lys104 and Lys147, acetylation has been detected at the N-terminus of RAS proteins. Combined mass spectrometric and structural analyses suggest that N-terminal acetylation stabilizes the N-terminal region and switch domains and may thereby contribute to RAS conformational integrity. N-terminal processing involving Thr2 acetylation has further been associated with stabilization of KRAS structure and maintenance of appropriate coupling to Mg^{2+}-dependent nucleotide binding [71].

4.4. Nitrosylation-mediated regulation of RAS signaling

S-nitrosylation provides a redox-dependent mechanism for regulating RAS activity. At Cys118, a residue conserved across HRAS, NRAS, and KRAS, NO-derived reactive nitrogen species generated downstream of iNOS and eNOS can promote the formation of transient thiyl radical intermediates. These intermediates disrupt GDP binding and facilitate nucleotide exchange, thereby modulating the transition between inactive and active RAS states [72]. Evidence indicates that this regulation is mediated primarily by transient radical intermediates formed during the nitrosylation reaction rather than by the stable S-nitrosylated Ras (Ras-SNO) product itself [73]. NO donors such as GSNO can facilitate this process through radical-mediated mechanisms and may also sustain compartmentalized Ras activation through a Src-PLCγ-Ca^{2+} cascade [74,75]. The source and downstream consequences of NO-mediated RAS regulation vary with cellular context. During inflammation, cytokine-induced iNOS-derived NO promotes RAS S-nitrosylation at Cys118 and activates the PI3K-Akt-mTOR pathway, enhancing protein translation and increasing iNOS protein levels in a positive feedback loop [76]. In tumors, oncogenic signaling appears to engage a distinct NO-dependent circuit. Oncogenic Ras activates PI3K-AKT signaling, which promotes phosphorylation of eNOS at Ser1177 and increases NO production. eNOS-derived NO then selectively induces S-nitrosylation of wild-type HRas and NRas at Cys118, increasing GTP loading and downstream signaling, whereas oncogenic Ras mutants are not affected [77].

4.5. Glycosylation in RAS regulation and oncogenic signaling

O-linked glycosylation of intracellular proteins includes two distinct modifications: pathogen-mediated O-glucosylation and reversible O-GlcNAcylation. Although both modifications occur on intracellular proteins, they exert fundamentally different biological effects on Ras signaling. Pathogen-mediated O-glucosylation is catalyzed by bacterial toxins such as *Clostridium sordellii* lethal toxin (TcsL), installs glucose on the conserved Thr35 residue within the switch I domain of Ras, locking it in an inactive GDP-bound state. Similarly, the clostridial toxin TpeL catalyzes O-GlcNAcylation of the same Thr35 residue, disrupting Ras–Raf interaction and consequently suppressing downstream MAPK signaling [78–80]. Beyond pathogen-mediated modifications, oncogenic Ras signaling also exploits endogenous O-GlcNAcylation pathways. Mutant KRAS enhances glucose and glutamine flux into the hexosamine biosynthetic pathway (HBP), resulting in increased production of UDP-GlcNAc and a global elevation of intracellular protein O-GlcNAcylation [81]. Unlike direct modification of RAS, O-GlcNAc glycosylation primarily functions as a downstream effector mechanism of RAS oncogenic signaling. It can enhance RAS-driven oncogenic programs by stabilizing key transcriptional and signaling regulatory factors such as Snail1 and c-MYC [82]. Under hyperglycemic conditions, KRAS mutations can promote O-GlcNAc glycosylation of the tight junction protein CLDN18.2 at its conserved C-terminal threonine site. This modification promotes the retention of CLDN18.2 in the cytoplasm and weakens its interaction with membrane scaffold proteins, leading to abnormal Src activation. In pancreatic cancer, this process can enhance tumor invasion and metastasis, and promote resistance to targeted therapy [83]. Overall, unlike pathogen-mediated glycosylation which can directly inhibit RAS activity, endogenous O-GlcNAc glycosylation primarily amplifies RAS-driven oncogenic signals by regulating multiple downstream effector molecules.

4.6. SUMOylation regulates KRAS effector engagement

Lys42 is a key SUMOylation site of KRAS. Replacing this site with arginine (KRAS V12/R42) can significantly inhibit the SUMOylation of KRAS. At the same time, overexpression of the SUMO-specific protease SENP1 can also reduce the level of SUMO-modified KRAS and weaken the interaction between KRAS and c-RAF. Consistent with this notion, oncogenic KRAS V12 displays substantially higher levels of SUMOylation than wild-type (WT) KRAS, which is accompanied by enhanced activation of downstream signaling pathways. Importantly, pharmacological inhibition of SUMOylation produces similar effects. Treatment of KRAS V12 pancreatic cancer cells with the SUMO E2 inhibitor 2-D08 markedly reduces KRAS SUMOylation, suppresses ZEB1 expression, and induces ZO-1 in a concentration-dependent manner, indicative of EMT reversal. In contrast, 2-D08 has little effect on BxPC-3 cells harboring WT KRAS, suggesting that KRAS-mutant tumors are preferentially dependent on SUMOylation for maintaining oncogenic signaling and malignant phenotypes. Collectively, these findings identify KRAS SUMOylation as a potential therapeutic vulnerability in KRAS-driven cancers [84].

5. PTMs regulating protein stability and degradation

RAS protein stability and signalling output are governed by a dynamic equilibrium between ubiquitination and deubiquitination (Figure 4). Multiple E3 ubiquitin ligases regulate RAS abundance through proteasomal degradation, including β-TrCP, LZTR1, NEDD4, WDR76 and SMURF2, whilst RABGEF1 (RABEX5) mono- and di-ubiquitinates HRAS and NRAS to reroute them to endosomal compartments, thereby attenuating MAPK output [85,86].

5.1. Dynamic ubiquitination controls RAS stability and signaling

E3 ligase-mediated ubiquitination of RAS regulates signaling through two functionally distinct mechanisms: non-degradative ubiquitination that potentiates oncogenic activity and degradative ubiquitination that limits RAS abundance and signaling output. In the non-degradative category, FBXL6 catalyses K63-linked polyubiquitination of KRAS at K128 to potentiate oncogenic signalling in hepatocellular carcinoma, K128R substitution attenuates this effect on both wild-type KRAS and KRAS G12D [87]. Conversely, several E3 ligases suppress tumour progression through targeted Ras degradation. The CUL4-DDB1 adaptor WDR76 induces polyubiquitination-dependent proteasomal degradation of all major Ras isoforms, attenuating MAPK signalling [88,89]. This axis can be pharmacologically engaged by kurarinone to degrade KRAS independently of p53 status, and correlates with 5-fluorouracil sensitivity in colorectal cancer [90,91]. Likewise, LZTR1-CUL3-mediated ubiquitination introduces a spatial layer of RAS regulation. Ubiquitination at HRAS K170 sequesters the farnesyl and palmitoyl anchors within the hypervariable region, limiting membrane association and downstream signaling. Accordingly, LZTR1 deficiency or mutation of HRAS Lys170 increases membrane association and enhances RAS pathway activation [92–95]. The functional consequences of RAS ubiquitination are, however, highly context dependent. Nedd4-1-mediated ubiquitination normally restrains RAS signaling through degradative control, however, this constraint is selectively bypassed in oncogenic or growth factor-driven contexts. In tumor cells, Nedd4-1 activity is redirected toward degradation of the tumor suppressor PTEN rather than RAS, thereby reinforcing PI3K-Akt signaling and

oncogenic output [96]. Additional E3 ligases further diversify Ras control through trafficking and competitive stabilization mechanisms. Rabex-5, acting as a dual-function GEF and E3 ligase, mono- and di-ubiquitinates HRAS and NRAS to retain them on early endosomes, thereby restricting plasma membrane signaling [97,98]. β-TrCP promotes polyubiquitination and degradation of HRAS, a process antagonized by Wnt signaling [99]. Tyr4 phosphorylation licenses Rabex-5 mediated ubiquitination, while GSK3β-mediated phosphorylation promotes β-TrCP dependent proteasomal degradation, a pathway suppressed by Wnt/β-catenin signaling in colorectal tumorigenesis [100,101]. In parallel, KRAS stability can be indirectly enhanced by AIMP2-DX2, which blocks SMURF2-dependent ubiquitination [102], or by the SMURF2-UBCH5 complex, which stabilizes KRAS by promoting degradation of β-TrCP1 [103].

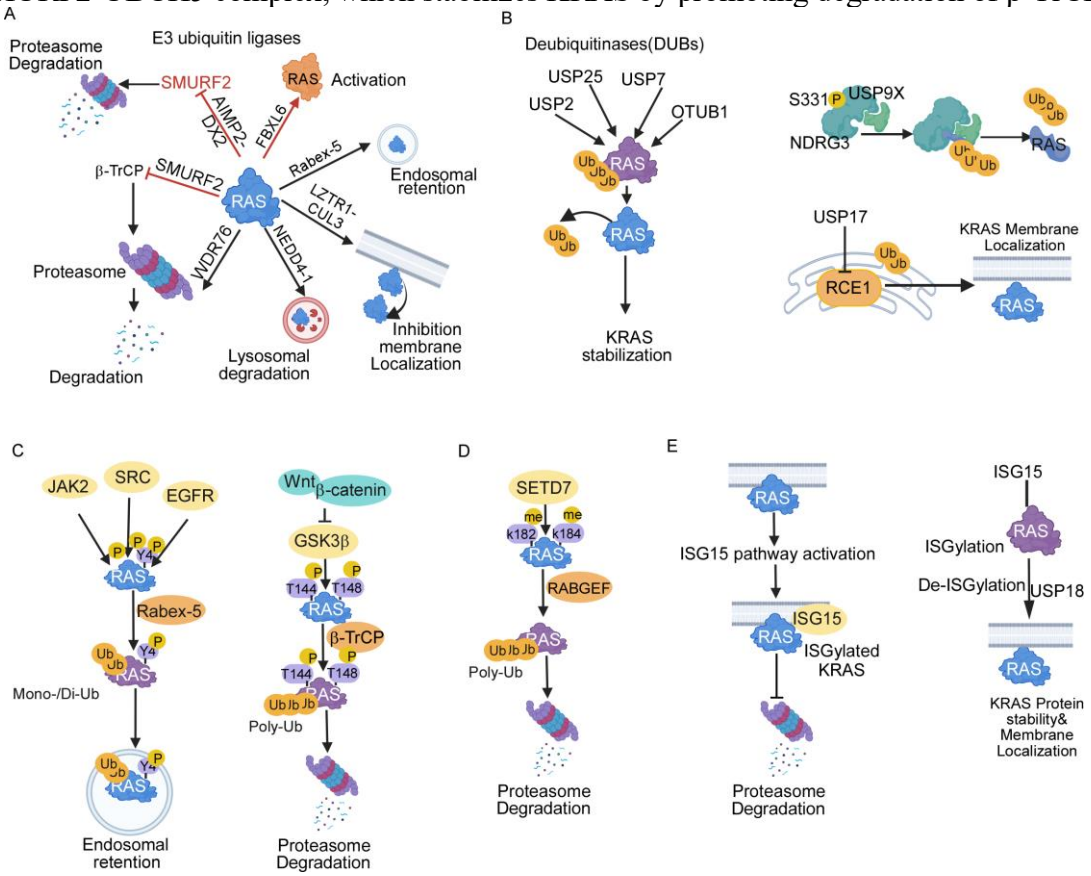

Figure 4. Ubiquitination, deubiquitination, and PTM-mediated regulation of RAS stability and degradation. **(A)** E3 ubiquitin ligases regulate RAS stability and trafficking; **(B)** Deubiquitinases (DUBs) stabilize RAS signaling; **(C)** Phosphorylation-dependent control of RAS ubiquitination and trafficking; **(D)** Methylation cooperates with ubiquitination to promote RAS degradation; **(E)** ISGylation regulates KRAS stability and membrane localization. Abbreviations: DUB, deubiquitinase; ISG15, interferon-stimulated gene 15; RCE1, Ras-converting enzyme 1; LZTR1, leucine zipper-like transcription regulator 1; CUL3, Cullin 3; RABGEF, Rab guanine nucleotide exchange factor; Ub, ubiquitin; P, phosphorylation; me, methylation.

Counteracting E3 ligase-mediated ubiquitination, deubiquitinases (DUBs) preserve RAS signaling by reversing ubiquitin-dependent degradation and regulating multiple steps of RAS activation. Several DUBs directly stabilize RAS proteins by removing degradative ubiquitin chains. Among these, USP7 selectively removes K48-linked ubiquitin from KRAS at K147, stabilizing the protein and promoting non-small cell lung cancer (NSCLC) progression [104]. Similarly, USP25 maintains KRAS abundance

by limiting KRAS ubiquitination and proteasomal degradation. It is worth noting that the ubiquitination mediating RAS degradation mainly occurs at lysine sites in HVR, such as K172 in KRAS4B and K169 in KRAS4A. This is different from the monoubiquitination events involved in signal regulation within the core domain [105]. Consistent with this stability regulation, USP2 can also act as a deubiquitinating enzyme to maintain RAS protein stability. In multiple myeloma, the role of USP2 further illustrates that RAS stability is actively regulated, while also suggesting that this process has potential therapeutic value [106]. In addition to directly deubiquitinating RAS, DUBs also regulate RAS signaling indirectly by controlling its post-translational processing and membrane trafficking. USP17 can deubiquitinate and inhibit the function of RCE1. RCE1 is an endopeptidase located in the endoplasmic reticulum, involved in the processing of the CAAX motif during RAS maturation. Therefore, USP17 can selectively interfere with the plasma membrane localization of HRAS and NRAS, and weaken MAPK and PI3K signaling, while its impact on KRAS4B is relatively minor. Further studies have found that RCE1 isoform 2 can undergo K63-linked ubiquitination at the K43 site, and USP17-mediated deubiquitination can regulate its subcellular localization and enzymatic activity [107–109]. OTUB1 is a non-classical regulator of RAS ubiquitination. It can bind to RAS proteins and inhibit RAS monoubiquitination and polyubiquitination without relying on its own catalytic activity. Its primary mechanism is not the direct removal of ubiquitin from RAS, but rather the reduction of RAS ubiquitination by inhibiting E2 ubiquitin-conjugating enzymes. After the reduction in ubiquitination levels, the translocation of RAS from the plasma membrane to the endomembrane system is restricted, thus retaining more RAS on the plasma membrane and enhancing downstream MAPK-ERK signaling. This effect is more pronounced in the wild-type RAS background, promoting the transformation of lung cancer cells and tumor growth [110]. In addition, scaffold protein-mediated recruitment can also enhance the specificity of regulation. For example, NDRG3 can promote the binding of KRAS to USP9X in a phosphorylation-dependent manner [111]. USP18 helps maintain the stability and membrane localization of KRAS and may link ubiquitin and ubiquitin-like modification pathways [112].

5.2. Methylation regulates RAS stability and protein interactions

Recent evidence has identified lysine methylation at sites such as Lys-5 and Lys-147 within the core GTPase domain of RAS, suggesting that methylation is also a post-translational regulatory mechanism of RAS. Notably, Lys5 methylation does not alter RAS conformation or nucleotide binding dynamics, but instead likely modulates signaling by reshaping protein-protein interactions or generating methyl-lysine recognition sites [113]. Additionally, SETD7 can mediate the methylation of KRAS at K182 and K184 in the C-terminal polybasic domain. This modification promotes the binding of KRAS to the E3 ubiquitin ligase RABGEF1, thereby inducing K11 and K48-linked polyubiquitination and facilitating its degradation via the proteasome. This can reduce the stability of KRAS and inhibit downstream RAS/MEK/ERK and PI3K/AKT signaling, thereby limiting the progression of NSCLC [114].

5.3. ISGylation stabilizes oncogenic KRAS signaling

Interferon-stimulated gene 15 (ISG15) is a ubiquitin-like modifier induced by interferon signaling. Following maturation, ISG15 can be covalently conjugated to substrate proteins through ISGylation [115–118]. This modification is mediated by an enzymatic cascade comprising the E1 enzyme UBE1L, the E2 enzyme

UBE2L6 (UbcH8), and E3 ligases including HERC5 [119,120]. In contrast to classical ubiquitination, which often targets proteins for proteasomal degradation, ISGylation can regulate protein stability, trafficking, and signal transduction and may also modulate ubiquitin-dependent pathways [121]. Burks *et al.* reported reciprocal regulation between oncogenic KRAS and the ISG15 pathway. Oncogenic KRAS increased ISG15 expression and global ISGylation, whereas enhanced ISGylation increased KRAS stability by limiting its lysosomal degradation. This reciprocal regulation was proposed to form a positive feedback loop that sustains oncogenic KRAS signaling. Knockdown of ISG15 or its E2 enzyme UbcH8 significantly reduced several KRAS-driven phenotypes, including cell proliferation, colony formation, anchorage-independent growth, epithelial-mesenchymal transition (EMT), and cell migration. The study did not demonstrate direct ISGylation of KRAS or identify specific KRAS modification sites. Instead, it linked the ISG15 pathway to the maintenance of oncogenic KRAS signaling and provided a basis for subsequent studies examining whether KRAS itself undergoes direct ISGylation [122].

The oncogenic activity of KRAS is shaped not only by genetic mutations but also by post-translational regulation. Different KRAS genotypes can be associated with distinct proteoform compositions and post-translational modification profiles, which may influence KRAS activation state, membrane binding capacity, and downstream signaling. For example, S-nitrosylation of Cys118 and C-terminal carboxymethylation show substantial dependence on the mutational background, suggesting that KRAS mutations can alter the functional consequences of specific post-translational modifications. Proteoform-level analysis of both wild-type and mutant KRAS may therefore provide a more detailed view of KRAS signaling heterogeneity and help clarify how distinct KRAS states relate to tumor staging, disease progression, and clinical outcomes [123].

6. PTMs creating direct therapeutic vulnerabilities

6.1. Progress in RAS targeted therapeutic development

Once considered “undruggable” RAS has undergone a remarkable therapeutic transformation since the 2013 discovery of the switch-II allosteric pocket in KRAS G12C. The covalent KRAS G12C inhibitors sotorasib and adagrasib (FDA-approved in 2021 and 2022) established proof of concept in NSCLC, and the 2025 approval of sotorasib plus panitumumab validated vertical pathway combinations in colorectal cancer. The arsenal has since expanded to include KRAS G12D and other allele-selective inhibitors, RAS(ON) tri-complex molecular glue inhibitors (e.g., daraxonrasib, which nearly doubled overall survival in the phase 3 RASolute 302 trial in pancreatic cancer), pan-KRAS/pan-RAS inhibitors, RAS degraders, and pan-RAS antibody drug conjugates (Figure 5, Table 1).

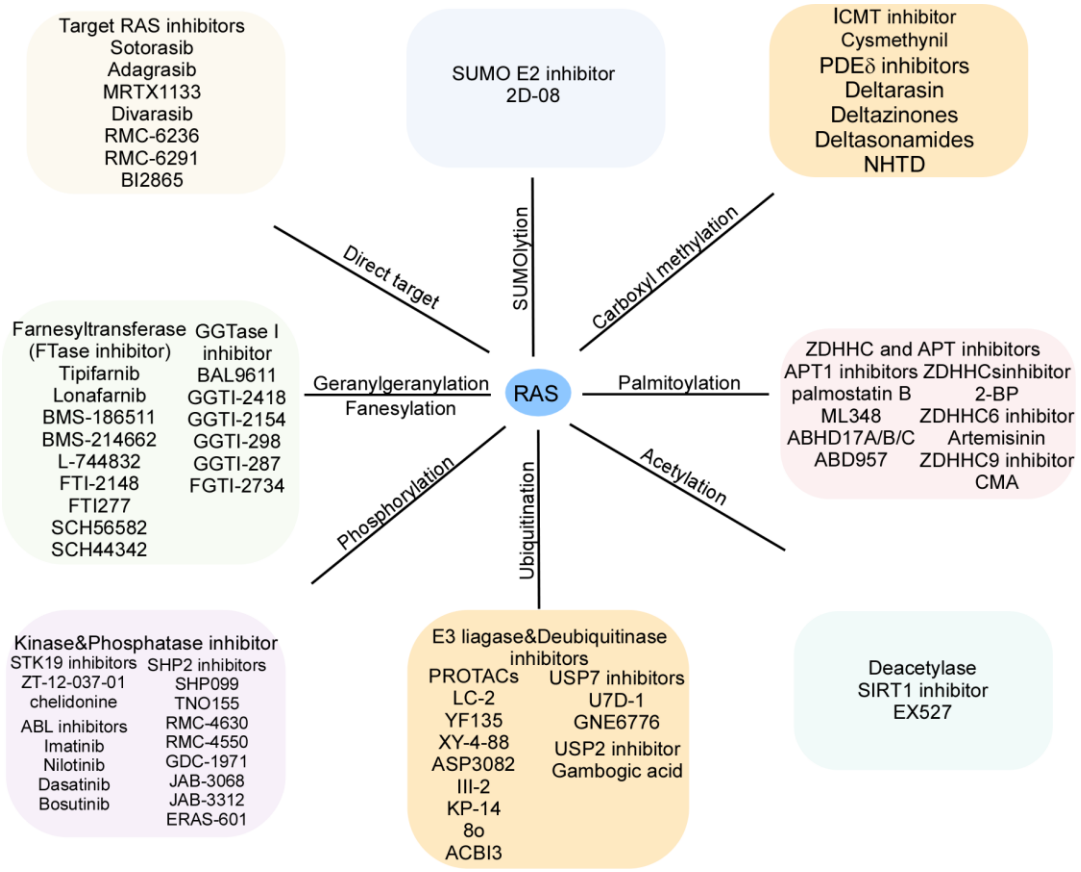

Figure 5. Therapeutic opportunities targeting RAS PTM networks. Multiple pharmacological strategies have been developed to directly inhibit RAS or to indirectly suppress RAS signaling by targeting enzymes responsible for its PTMs.

Table 1. Clinically developed single agent inhibitors

Target	Development stage	Agent	Representative indication (s)	Representative trial (s)
KRASG12C	Approved	Sotorasib (AMG 510)	NSCLC; other solid tumors	NCT03600883
	Approved	Adagrasib (MRTX849)	NSCLC; CRC; other solid tumors	NCT04613596
	Late-stage clinical	Glecirasib (JAB-21822)	NSCLC; CRC; pancreatic cancer; other solid tumors	NCT05009329; NCT05288205
	Late-stage clinical	LY3537982	NSCLC; CRC; pancreatic and other solid tumors	NCT04956640
	Late-stage clinical	IBI-351	NSCLC; CRC; solid tumors	NCT05005234
	Early-stage clinical	Garsorasib (D-1553)	NSCLC; CRC; solid tumors	NCT04585035
		Fulzerasib	Lung cancer	NCT06936644
		Divarasib	NSCLC; CRC; solid tumors	NCT04449874
		JDQ443	NSCLC; CRC; solid tumors	NCT04699188
		RMC-6291	NSCLC; CRC; pancreatic cancer	NCT05462717
		FMC-376	NSCLC; CRC; pancreatic and other solid tumors	NCT06244771
		HRS-7058	Advanced solid tumors	NCT06383871
		GH35	NSCLC; CRC; solid tumors	NCT05010694
		ZG-19018	Solid tumors	NCT06237400
		HYP-2090PTSA	Advanced solid tumors	NCT06243354

Table 1. *Cont.*

Target	Development stage	Agent	Representative indication (s)	Representative trial (s)
		HS-10370	NSCLC; CRC; solid tumors	NCT05367778
		BBO-8520	Advanced/metastatic NSCLC	NCT06343402
		GEC255	Advanced solid tumors	NCT05877654
		D3S-001	Solid tumors	NCT05410145
		BPI-421286	Advanced/metastatic solid tumors	NCT05315180
		BI 1823911	Solid tumors	NCT04973163
		JNJ-74699157	NSCLC; CRC; solid tumors	NCT04006301
KRASG12D	Early-stage clinical	MRTX1133	PDAC; NSCLC; CRC; other solid tumors	NCT05737706
		RMC-9805	PDAC; NSCLC; CRC; solid tumors	NCT06040541
		HRS-4642	Solid tumors	NCT05533463
		TSN1611	Advanced malignancies	NCT06385925
		AZD0022	NSCLC; PDAC; CRC; solid tumors	NCT06599502
		INCB161734	Solid tumors	NCT06179160
		LY3962673	PDAC; NSCLC; CRC	NCT06586515
		QLC1101	NSCLC; CRC; pancreatic cancer	NCT06403735
		PT0253	Solid tumors	NCT06797336
		GDC-7035	Solid tumors	NCT06619587
		ASP4396	Solid tumors	NCT06364696
		ASP3082	Solid tumors	NCT01958047
	Preclinical/emerging	ERAS-4	Solid tumor models	NCT03757455
		QTX3046	Tumor models	NCT06227377
Pan-RAS	Late-stage clinical/registrational	Daraxonrasib (RMC-6236)	PDAC; RAS-mutant NSCLC; other solid tumors	NCT05379985
	Early-stage clinical	YL-17231	Solid tumors	NCT06078800
		ERAS-0015	Metastatic solid tumors	NCT06983743
Pan-KRAS	Early-stage clinical	QTX3034	Solid tumors	NCT06227377
		LUNA18	Solid tumors	NCT05012618
		BI 3706674	Solid tumors; gastroesophageal cancers	NCT06056024
		ERAS-4001	Metastatic solid tumors	NCT07021898
		ALTA3263	PDAC; NSCLC; CRC; solid tumors	NCT06835569
		LY4066434	PDAC; NSCLC; CRC; solid tumors	NCT06607185
		PF-07934040	PDAC; CRC; NSCLC	NCT06447662
		BGB-53038	CRC; NSCLC; PDAC; gastroesophageal cancers	NCT06585488
		QTX3544	Advanced solid tumors	NCT06715124
		JAB-23E73	Advanced solid tumors	NCT06959615; NCT06973564

6.1.1. Targeting KRASG12C mutation

Two covalent KRAS G12C inhibitors, sotorasib (Lumakras; Amgen) and adagrasib (Krazati; Mirati Therapeutics/Bristol Myers Squibb), have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of KRAS G12C-mutant cancers [124–127]. Both compounds exploit the mutant cysteine residue (Cys12) by incorporating an electrophilic warhead that irreversibly binds the Switch II pocket, thereby trapping KRAS in its inactive GDP-bound state. Sotorasib contains an acrylamide warhead whose non-covalent scaffold extends deeply into the Switch II pocket and forms stabilizing interactions with residues including His95, Tyr96, and Gln99. Clinical evidence supporting KRAS G12C inhibition has been established across multiple trials. In the phase I/II CodeBreaK 100 study, the confirmed objective response rate (ORR) of sotorasib was 32.2%, and the disease control rate reached 88.1%. The median progression-free survival (PFS) was 6.3–6.8 months, and the median overall survival (OS) was 12.5 months. Based on these results, sotorasib received accelerated approval from the FDA for KRAS G12C-mutated NSCLC [127,128]. The subsequent Phase III CodeBreaK 200 study further confirmed its clinical benefits and supported its use as an important second-line treatment option for previously treated KRAS G12C mutation-positive NSCLC patients [129]. In contrast, the monotherapy efficacy of KRAS G12C inhibitors in CRC is relatively limited. The ORR of sotorasib monotherapy is usually less than 10%, with a median PFS of only about 2–4 months. After combining with the anti-EGFR antibody panitumumab, the efficacy significantly improved, with the ORR increasing to approximately 30%–35% and the median PFS extending to about 5–6 months. These results indicate that EGFR-mediated feedback reactivation is an important mechanism for primary resistance of CRC to KRAS G12C inhibitors, and also support the idea that vertically blocking the same signaling pathway can enhance therapeutic efficacy [130]. In addition to NSCLC and CRC, sotorasib is also undergoing Phase II studies in pancreatic cancer, appendiceal cancer, and biliary tract tumors. Overall efficacy is limited, but some patients can achieve a more durable response. Nevertheless, acquired resistance is almost inevitable. Its mechanisms are quite complex, including secondary KRAS mutations such as Y96D, H95Q/R/P, and Q61H, MET amplification, EGFR reactivation, and bypass signal activation mediated by BRAF or PIK3CA mutations, additionally, histological transformation can also contribute to the development of resistance [131–134].

Adagrasib is also a covalent KRAS G12C inhibitor that can covalently bind to Cys12 through a chloroacetamide electrophilic group. Compared to sotorasib, its non-covalent binding mechanism is different, as it can interact with different residues in the Switch II pocket, such as Met72, resulting in certain differences in conformational stability and pharmacokinetic characteristics. In CRC, the efficacy of adagrasib as a monotherapy remains limited. The Phase III KRYSTAL-10 study showed that the efficacy significantly improved after combining with the EGFR monoclonal antibody cetuximab. In previously treated patients, adagrasib has produced an objective response rate (ORR) of 30%-35% and a median PFS of approximately 5-6 months [135]. Reported resistance mechanisms broadly overlap with those of sotorasib and include secondary KRAS mutations and activation of bypass signaling pathways. In KRAS G12C-mutated NSCLC, further clinical evidence has come from the phase III KRYSTAL-12 study, in which adagrasib prolonged PFS relative to docetaxel in previously treated patients. The median PFS was approximately 6.8-7.2 months, with an ORR of 43%-46% and a median OS close to 12 months [136].

Efforts to improve the efficacy and resistance profile of first-generation KRAS G12C inhibitors have focused on optimizing target engagement, central nervous system exposure, and activity against acquired resistance. Many of these agents continue to target the Switch II pocket and stabilize KRAS in the GDP-bound inactive state, although structural modifications can alter their pharmacological properties. JDQ443 is a next-generation covalent inhibitor designed around the Switch II pocket, with additional interactions involving residues such as Arg68. In phase I/II studies, JDQ443 showed activity in NSCLC, with an ORR of 43.2%, a disease control rate (DCR) of 86.4%, and a median PFS of 5.6 months. By contrast, the ORR in CRC was 6.9%, indicating substantial variation in activity across tumor types [137]. JAB-21822 similarly produced an ORR of 53.6%, a DCR of 92.9%, and a median PFS of 8.3 months in NSCLC. Preliminary evidence also suggests central nervous system penetration, which may be relevant in patients with central nervous system metastases. GDC-6036, which has entered phase III evaluation, achieved an ORR of 35.3% (12/34), a DCR of 88.2%, and a median PFS of 7.6 months in early clinical studies (NCT04449874), supporting continued evaluation of structurally optimized covalent KRAS G12C inhibition [138,139]. LY3537982 is another next-generation KRAS G12C inhibitor that binds the Switch II pocket and stabilizes inactive KRAS. In NSCLC, LY3537982 achieved an ORR of 38.5%, a DCR of 92.3%, and a median PFS of 8.2 months in NCT04956640. Several additional covalent KRAS G12C inhibitors are also in early clinical development, including D3S-001 (NCT05073127) [140], BBO-8582 (NCT06343402), and GH35 (NCT05010694), with early studies reporting acceptable safety profiles, favourable pharmacokinetics, and preliminary antitumour activity. These next-generation KRAS G12C inhibitors reflect a shift from initial proof-of-concept targeting toward optimization of binding interactions, tissue distribution, and resistance coverage. Durable disease control remains limited in some settings, particularly CRC, and adaptive pathway reactivation continues to complicate treatment. These limitations provide a rationale for combination strategies and for agents capable of targeting a broader range of KRAS conformational and resistance states.

6.1.2. KRASG12D inhibitors

KRAS G12D accounts for approximately 28% of all KRAS mutations and is one of the most common KRAS mutations in CRC and pancreatic ductal adenocarcinoma (PDAC). Unlike KRAS G12C, the G12D mutation replaces the 12th glycine with aspartic acid, lacking a thiol group for covalent binding, making it difficult to employ the covalent targeting strategies relied upon by G12C inhibitors. Based on this characteristic, the development of KRAS G12D drugs mainly focuses on non-covalent Switch II pocket inhibitors, allosteric inhibitors, and strategies that achieve selective binding through protein complexes [141].

MRTX1133 is one of the earlier representative molecules to directly inhibit KRAS G12D. This compound is further optimized from the adagrasib-related scaffold, using a pyrido[4,3-d]pyrimidine core structure, and can bind with high affinity to the Switch II pocket, with a dissociation constant (KD) of approximately 0.2 pM [142,143]. MRTX1133 entered Phase I clinical trials (NCT05737706) in 2023, demonstrating the feasibility of directly targeting KRAS G12D. However, this class of compounds generally has the issue of limited oral bioavailability, so pharmacokinetic optimization has become an important direction for subsequent development. To overcome these limitations, subsequent drugs began to adopt different structures and mechanisms of action. Zoldonrasib (RMC-9805) employs the ternary complex strategy from Revolution Medicines. It does not merely occupy the Switch II pocket directly,

but rather forms a complex that selectively binds to the GTP state of KRAS G12D by recruiting cyclophilin A (CypA). Early clinical results showed that in the Phase I RMC-9805-001 study (NCT06040541), the objective response rate (ORR) for KRAS G12D mutant NSCLC patients reached 61%, the disease control rate (DCR) was 89%, and the median time to response was 1.4 months. Based on these results, RMC-9805 received FDA breakthrough therapy designation for previously treated KRAS G12D mutant NSCLC patients [144]. At the same time, traditional non-covalent inhibitors are also being continuously optimized. HRS-4642 is a high-affinity, long-acting KRAS G12D inhibitor [145,146]. In advanced KRAS G12D mutant PDAC, HRS-4642 combined with gemcitabine and nab-paclitaxel showed good therapeutic activity, with an ORR of 63.3%, a DCR of 93.3%, a 6-month progression-free survival rate of 89.3%, and overall manageable safety. Other candidate drugs based on structural optimization are also being advanced. ERAS-5024 replaces the pyrido[2,3-d]pyrimidine core of MRTX1133 with a quinazoline scaffold and introduces a 2-amino-3-cyanobenzothiophene moiety, thereby increasing hydrogen bonding interactions with Glu63 and Asp69, and enhancing *in vivo* antitumor activity [147]. AZD0022 is an oral analog of MRTX1133, primarily used to improve the pharmacokinetic characteristics of the original compound while retaining strong target inhibition capabilities. Currently, the drug is undergoing evaluation for monotherapy and combination therapy in Phase I/IIa studies. Overall, the development of KRAS G12D drugs has gradually progressed from early concept validation to the clinical optimization stage. Current strategies not only focus on inhibitory efficacy but also place greater emphasis on oral exposure, pharmacokinetic characteristics, duration of efficacy, and mechanisms of action at different targets. In addition to the aforementioned drugs, GFH375/VS-7375 (NCT06500676), INCB161734 (NCT06179160) [148], LY3962673 (NCT06586515), TSN1611 (NCT06385925), QLC1101 (NCT06403735), and These advancements indicate that the drug development for KRAS G12D is rapidly expanding and gradually forming multiple parallel therapeutic strategies.

6.1.3. Pan-(K)RAS inhibitors

The emergence of acquired resistance to mutation-selective KRAS inhibitors has shifted considerable attention toward broader targeting strategies capable of suppressing diverse KRAS alleles and preventing adaptive pathway reactivation. Resistance commonly arises through secondary KRAS mutations, reactivation of wild-type KRAS, or compensatory signaling mediated by alternative RAS isoforms, providing a strong rationale for the development of pan-KRAS and pan-RAS inhibitors [149–152]. Two complementary approaches have emerged. One exploits cyclophilin A (CypA)-mediated tricomplex formation to selectively engage the active, GTP-bound state of RAS proteins. AN9025 has been designed as an orally available pan-RAS(ON) inhibitor that stabilizes a high-affinity RAS-CypA tricomplex, producing potent and durable antitumor activity in preclinical RAS-mutant models.

Daraxonrasib (RMC-6236) is a RAS(ON) multi-selective molecular glue that recruits cyclophilin A to GTP-bound RAS, forming a synthetic ternary complex that disrupts RAS-effector interactions across multiple mutant and wild-type RAS proteins [153]. Preclinical studies indicate that sensitivity is influenced by RAS-GTP abundance and tumour dependence on active RAS, and clinical proof of concept has been established in previously treated RAS-mutant pancreatic ductal adenocarcinoma (PDAC), with second-line treatment producing an objective response rate of 35% and a median progression-free survival of 8.5 months in patients with RAS G12-mutant disease [154,155]. Nevertheless, resistance can arise through disruption of molecular-glue activity, including KRAS Y64 or Y71 and BRAF alterations,

or through restoration of oncogenic signalling; notably, amplification of the pre-existing mutant KRAS allele emerged in 36% of daraxonrasib-treated patients with PDAC who developed acquired resistance, alongside RTK-MAPK and PI3K pathway alterations [156,157]. Persistent RHEB-mTORC1 signalling and treatment-induced EMT and stromal remodelling further illustrate parallel and non-genetic routes of adaptation [158,159]. Together, these findings suggest that durable responses to broad RAS(ON) inhibition will require not only deep RAS suppression but also mechanism-guided combinations that intercept pathway reactivation and adaptive bypass signalling [160–162].

A second strategy focuses on direct inhibition of KRAS irrespective of mutation subtype while minimizing activity against HRAS and NRAS to improve tolerability. LY4066434 and ERAS-4001 represent this emerging class of pan-KRAS inhibitors, displaying activity against both mutant and wild-type KRAS while largely sparing other RAS family members. Notably, ERAS-4001 binds wild-type KRAS and multiple KRAS G12X variants with single-digit nanomolar affinity and exhibits marked selectivity over HRAS and NRAS, suggesting the potential for a wider therapeutic window. Complementing these approaches, BBO-11818 employs a distinct non-covalent mechanism by engaging both GDP- and GTP-bound KRAS, stabilizing an inactive conformation that disrupts effector interactions, including RAF1, and thereby suppresses downstream oncogenic signaling. Collectively, these next-generation inhibitors reflect a broader transition from mutation-restricted targeting toward allele-agnostic inhibition of KRAS or RAS signaling. By expanding target coverage while addressing common mechanisms of adaptive resistance, pan-KRAS and pan-RAS inhibitors may provide a more durable therapeutic strategy for RAS-driven malignancies, although balancing broad pathway inhibition with acceptable tolerability remains a key challenge for clinical development.

6.2. Therapeutic targeting of KRAS post-translational modifications

6.2.1. Targeting lipid modifications

Farnesylation of RAS by farnesyltransferase (FTase) is the first irreversible step in CaaX processing and is required for efficient membrane association [163]. The response to FTase inhibitors (FTIs) differs among RAS isoforms. KRAS and NRAS can bypass FTase inhibition through alternative geranylgeranylation by GGTase-1, whereas HRAS depends predominantly on farnesylation for membrane association. This isoform-specific difference underlies the clinical activity of tipifarnib in HRAS-mutant head and neck squamous cell carcinoma [164]. The FTIs SCH 56582 and SCH 44342 suppress HRAS prenylation-dependent membrane association, whereas KRAS and NRAS can retain membrane localization and partial downstream signaling through alternative geranylgeranylation, contributing to the limited efficacy of FTIs in KRAS-mutant tumors [165]. The FTI L-744832 has also been reported to affect signaling beyond direct inhibition of prenylation by downregulating DNMT1, restoring TGF-βRII-Smad activity, and enhancing radiosensitivity even in p53-deficient contexts [166]. RAS prenylation can also be perturbed indirectly through inhibition of the mevalonate pathway. By reducing cellular pools of farnesyl and geranylgeranyl pyrophosphate, statins attenuate RAS prenylation and signaling and have also been associated with endoplasmic reticulum stress and immunogenic cell death [167]. Because alternative prenylation can limit the activity of selective FTIs against KRAS-mutant tumors, dual FTase/GGTase-1 inhibitors have been developed. FGTI-2734 blocks both farnesylation and geranylgeranylation, thereby preventing KRAS membrane localization. This dual inhibition disrupts

KRAS interactions with RAF1 and other downstream effectors, suppresses the PI3K/AKT, mTOR, and ERK pathways, and promotes apoptosis through upregulation of p53 and suppression of c-MYC [168].

RAS membrane localization can also be targeted at the level of intracellular trafficking. Deltarasin, a small-molecule inhibitor of the PDE6δ-KRAS4B interaction, impairs KRAS4B accumulation at the plasma membrane and reduces the growth of KRAS-mutant tumor cells [169]. Adaptive signaling may also limit the durability of KRAS G12C inhibition. Although sotorasib and adagrasib suppress mutant KRAS G12C activity, treatment can induce compensatory activation of wild-type HRAS. Concurrent inhibition of farnesyltransferase has been reported to counteract this response. Combinations of sotorasib or adagrasib with tipifarnib or lonafarnib produced synergistic antitumor effects in KRAS G12C-mutant models, accompanied by further suppression of RHEB-mTOR signaling and cell-cycle progression [170].

Palmitoylation cycling provides another mechanism for controlling RAS localization. ABHD17-mediated depalmitoylation of NRAS is required for its redistribution from the plasma membrane to intracellular compartments. Selective ABHD17 inhibition with ABD957 maintains NRAS in a palmitoylated, membrane-bound state and significantly reduces ERK phosphorylation in NRAS-mutant leukemia and cancer cells. Combining ABD957 with the MEK inhibitor PD901 produces synergistic growth inhibition, suggesting that ABHD17 inhibition may enhance the activity of existing targeted therapies [33]. The enzymatic control of NRAS palmitoylation has also been investigated through both depalmitoylases and palmitoyltransferases. Early models assigned a dominant depalmitoylating role to APT1 and APT2, supported by pharmacological studies using palmostatin B. More recently, artemisinin (ART) has been shown to covalently interact with the palmitoyltransferase ZDHHC6, reducing its enzymatic activity and thereby decreasing NRAS palmitoylation. This loss of palmitoylation disrupts proper NRAS subcellular localization, redistributing NRAS from the Golgi apparatus to the cytoplasm and attenuating downstream ERK and AKT signaling pathways involved in cell proliferation and survival [171].

6.3. Targeting RAS kinases and phosphatases as an alternative therapeutic strategy

Targeting the kinases and phosphatases that regulate RAS activity is an important therapeutic strategy for directly inhibiting RAS. SHP2 is a non-receptor protein tyrosine phosphatase that can promote RAS/ERK/MAPK signaling. The highly selective allosteric inhibitor SHP099 can bind to the SH2-PTP domain interface, stabilizing SHP2 in an autoinhibited conformation, with an IC_{50} of 0.071 μM, and it shows minimal cross-inhibition with the homologous protein SHP1. Although SHP099 has limited inhibitory effect on the proliferation of KRAS or BRAF mutant cells *in vitro*, it can significantly inhibit tumor growth in KRAS mutant xenograft models [172]. Further studies show that the combined inhibition of SHP2 and MEK has a stronger anti-tumor effect in KRAS-mutant models of pancreatic cancer, ovarian cancer, and lung cancer, and can alleviate single-agent resistance to MEK inhibitors [173]. In clinical studies, the SHP2 inhibitor RMC-4630 achieved a 67% disease control rate in advanced KRAS-mutant non-small cell lung cancer. JAB-3068 and JAB-3312 are also being evaluated for safety and preliminary efficacy in KRAS-mutant solid tumors (NCT03565003, NCT04045496). In addition to SHP2, the serine/threonine kinase STK19 can also regulate RAS activity. STK19 can enhance the binding of NRAS to downstream effector proteins by phosphorylating the Ser89 site of NRAS. The STK19 inhibitor ZT-12-037-01 (1a) can inhibit the growth of NRAS-driven melanoma both *in vitro* and *in vivo*, and the subsequently discovered chelidonine also exhibited similar effects in preclinical

models [55,57]. In addition, the deubiquitinating enzyme USP2 is also a potential indirect RAS target. Gambogic acid can covalently bind to the allosteric site Cys284 of USP2, which is different from its catalytic site Cys276. This binding can induce conformational changes in USP2 and inhibit its activity, thereby increasing the ubiquitination of KRAS and promoting its proteasomal degradation. It can be seen that inhibiting USP2 can reduce RAS signaling by enhancing KRAS clearance [106].

6.4. E3 Ligases: leveraging the ubiquitin-proteasome system

Protein-targeting chimeric (PROTAC) technology, as a powerful strategy for targeted protein degradation, enables the pharmacological elimination of disease-associated proteins. A protein-targeting chimeric is a bifunctional small molecule composed of three structural elements: (1) a warhead that recognizes and binds the protein of interest (POI); (2) a recruitment element that binds to an E3 ubiquitin ligase; and (3) a linker connecting these two elements. By simultaneously engaging the protein of interest (POI) and an E3 ubiquitin ligase, a PROTAC promotes formation of a ternary complex that facilitates target polyubiquitination and subsequent degradation by the 26S proteasome. The von Hippel-Lindau (VHL) and Cereblon (CRBN) E3 ubiquitin ligase systems are among the most widely used in PROTAC design. Pharmacological degradation of oncogenic drivers, including mutant KRAS, has been shown to reduce RAS-dependent signalling and associated oncogenic phenotypes in preclinical models, providing a basis for evaluating targeted protein degradation in RAS-driven malignancies.

LC-2 was generated by linking the KRAS G12C inhibitor MRTX849 to a VHL E3 ligase ligand, extending KRAS-directed PROTAC degradation beyond earlier systems based on artificial fusion proteins such as GFP-KRAS G12C [174]. YF135, described as the first reversible covalent PROTAC derived from MRTX849, targets KRAS G12C and recruits VHL to induce degradation, with a reported DC_{50} of approximately 3-4 μM and accompanying suppression of p-ERK. The study reported no detectable hook effect and showed that degradation was reversible and VHL dependent, this design was proposed to address the reduced catalytic efficiency associated with irreversible PROTACs [175]. ZJK-807, a KRAS G12D-targeting PROTAC derived from an MRTX1133 analog and a CRBN ligand, selectively degrades KRAS G12D without affecting wild-type KRAS or other mutants. It also retains activity in models of MRTX1133 resistance, including Q95/Y96 alterations, suppresses RAS/MAPK signaling, and produced 47% tumor inhibition *in vivo* together with favorable pharmacokinetic properties [176].

Beyond direct small-molecule KRAS degraders, the Affinity-Directed Protein Missile (AdPROM) system has been investigated as an alternative strategy for directing RAS proteins to the proteasome. Expression of a VHL-αGFP AdPROM construct, in which VHL is fused to a GFP nanobody, induced near-complete proteasomal degradation of GFP-KRAS; degradation was rescued by MLN4924 and confirmed by flow cytometry. To target untagged endogenous RAS, a VHL-αHRAS AdPROM construct, comprising VHL fused to an HRAS monobody, achieved approximately 50% degradation of endogenous HRAS and approximately 15% degradation of KRAS in wild-type A549 cells without affecting NRAS, as assessed by Western blot and TMT proteomics. MG132 and bortezomib reversed this degradation, supporting dependence on the ubiquitin-proteasome system (UPS) [177].

Clinical development of KRAS-directed degraders has also begun. ASP3082 is a first-in-class (FIC) KRAS G12D-selective proteasome degradation agent that has demonstrated antitumor activity in PDAC patients in a Phase I study (NCT05382559). Reported overall response rates (ORRs) were approximately

8% among evaluable patients, approximately 19% in the pancreatic ductal adenocarcinoma cohort, and approximately 23% in the high-dose NSCLC group [178,179]. ASP4396 is a KRAS G12D-targeted protein degrader being evaluated in an open-label, Phase I dose-escalation study (NCT06364696) in patients with advanced solid tumors harboring KRAS G12D mutations, with safety, pharmacokinetics/pharmacodynamics (PK/PD), and preliminary antitumor activity as the primary objectives. ACBI3 extends this strategy across several common oncogenic KRAS mutations. Through non-covalent KRAS binding and VHL recruitment, ACBI3 induces target degradation and produces more sustained MAPK pathway inhibition than traditional inhibitors. It selectively kills KRAS-mutant cancer cells and significantly inhibits tumor growth *in vivo*, supporting further evaluation of mutation-agnostic degradation strategies for KRAS-driven cancers [180]. SHP2, a nonreceptor protein Tyr phosphatase, preferentially binds to and dephosphorylates RAS, stimulating downstream RAS/ERK/MAPK cascades. The highly selective allosteric inhibitor SHP099 binds to the SH2-PTP domain interface of SHP2, stabilizing the autoinhibited conformation with an IC50 of 0.071 μM, and shows no cross-reactivity with homologous proteins such as SHP1 [181]. Based on the PROTAC technology, a series of SHP2 degraders were designed and synthesized, and the candidate molecule SHP2-D26 was eventually identified. By optimizing the linker length (12–14 methylene groups) and composition (introducing a piperazine group) between the SHP2 inhibitor (compound 5) and the VHL ligand, SHP2-D26 exhibited strong degradation activity in KYSE520 and MV4;11 cells (DC50 = 6.0 nM and 2.6 nM), leading to the degradation of >95% of SHP2 protein. SHP2-D26 induces SHP2 degradation through the VHL-dependent ubiquitin-proteasome pathway and significantly blocks the RAS-ERK signaling pathway. Compared to the traditional inhibitor SHP099, SHP2-D26 enhances the inhibition potency of ERK phosphorylation by over 30 times and improves the inhibition of cell proliferation by 28–100 times [182].

7. Discussion and future perspectives

PTMs add a further regulatory layer to canonical RAS control by guanine nucleotide exchange factors and GTPase-activating proteins. Unlike the relatively well-defined GDP/GTP cycle, however, the functional consequences of RAS PTMs are highly context dependent. Rather than acting as simple binary switches, PTMs influence RAS conformation, membrane affinity, subcellular localization, effector engagement, and protein stability (Table 2). RAS PTMs should therefore be viewed as components of a dynamic regulatory network that integrates biochemical activity with spatial organization.

Table 2. Evidence, isoform specificity and functional consequences of post-translational modifications of RAS proteins.

PTM	Major Modification Site(s)	Direct evidence and experimental validation	Writer/Eraser	Reported RAS isoform(s)	Major Functional Consequences
Farnesylation	HRAS(C181/C186), NRAS (C186), KRAS4A (C186), KRAS4A (C185)	Directly established at the C-terminal CAAX cysteine by biochemical and enzymatic characterization.	Farnesyltransferase (FTase); GGTase I	HRAS, NRAS, KRAS4A, KRAS4B	Essential for membrane anchoring and plasma membrane localization
Palmitoylation	HRAS (C181/C184), NRAS (C181), KRAS4A (C180)	Directly established at HRAS C181/C184, NRAS C181 and KRAS4A C180 by biochemical/acylation assays and trafficking analyses.	ZDHHC family (e.g., ZDHHC9/GCP16); APT1/2, ABHD17 family	HRAS, NRAS, KRAS4A	Regulates membrane trafficking, Golgi–plasma membrane cycling, and spatial signaling
Carboxymethylation	Prenylated C-terminal cysteine	Directly established at the C-terminal prenylated cysteine by biochemical and enzymatic analyses.	ICMT	HRAS, NRAS, KRAS4A, KRAS4B	Enhances membrane affinity and stabilizes membrane association
Phosphorylation	Y4, Y32, Y64, S89, Y137 T144, T148, S181	Endogenous RAS phosphorylation demonstrated, with KRAS Y32/Y64 validated by MS/NMR; supported by phospho-IP/IB, kinase/phosphatase assays, mutagenesis, GTPase and effector-binding assays, and cellular/*in vivo* studies.	JAK2, EGFR, PKC, PKG, SRC, ABL, GSK3b, STK19, PTPN2, SHP2	HRAS, NRAS, KRAS4A, KRAS4B	Regulates membrane association, nucleotide cycling, and effector interactions
Ubiquitination	K5, K104, K117, K128, K147 and K170	Directly established at multiple sites, including KRAS K104, K128 and K147, by MS and chemically/ site-specifically ubiquitinated RAS.	E3 ligases (e.g., LZTR1, Rabex-5, NEDD4–1, b-TrCP. SMURF2, WDR76, FBXL6); DUBs (USP7, USP17, USP9X/NDRG3, USP18, USP25, USP2)	HRAS, NRAS, KRAS4A, KRAS4B	Controls protein stability, GTP loading, effector binding, and proteasomal degradation
Acetylation	K104, K147	Direct evidence, strongest for K104 and K147, from endogenous RAS analysis and MS; defined modified proteins have also been used in functional studies.	p300/CBP; HDAC6 and SIRT2, SIRT1	KRAS4A, KRAS4B	Modulates nucleotide exchange, conformational dynamics, and oncogenic fitness

Table 2. *Cont.*

PTM	Major Modification Site (s)	Direct evidence and experimental validation	Writer/Eraser	Reported RAS isoform (s)	Major Functional Consequences
Methylation	K5, K147, K182, K184	MS analysis of immunoprecipitated endogenous RAS supports K5 dimethylation and K147 monomethylation.	SETD7	KRAS4A, KRAS4B	Regulates protein stability
S-Nitrosylation	C118	Directly demonstrated at C118 by MS and biochemical/ structural analyses.	Nitric oxide (NO)-dependent modification	HRAS, NRAS, KRAS4A, KRAS4B	Promotes GDP dissociation and RAS activation
SUMOylation	K42, K104, K147	Evidence supports RAS SUMOylation, with K42 having the strongest functional support, primarily from IP/immunoblotting and *in vitro* SUMOylation assays.	SENP1	HRAS, NRAS, KRAS4A, KRAS4B	Regulates protein stability, localization, and signaling activity
Glycosylation/ O-GlcNAcylation	T35	Direct endogenous KRAS O-GlcNAcylation has not been convincingly established in major tumor studies; evidence mainly reflects alterations in O-GlcNAc pathways downstream of mutant KRAS.	TcsL and TpeL	KRAS4A, KRAS4B	Potentially regulates protein stability or subcellular localization
ADP-ribosylation	R41, R128	Direct RAS modification has been demonstrated by biochemical and cellular assays, predominantly in bacterial-toxin systems.	PARP/ART family	KRAS4A, KRAS4B	Disruputs effector binding and downstream signaling

A useful conceptual distinction can be made between PTMs within the G domain and those in the C-terminal hypervariable region (HVR). G-domain modifications, including ubiquitination, acetylation, SUMOylation, nitrosylation, and methylation, generally occur at relatively low stoichiometry and can influence nucleotide exchange, conformation, or effector binding. By contrast, CaaX-associated farnesylation, proteolysis, and carboxymethylation constitute constitutive maturation steps that establish membrane-binding competence. Reversible HVR modifications, including palmitoylation and phosphorylation, subsequently modulate membrane affinity and trafficking. RAS proteins traffic dynamically among the plasma membrane, Golgi apparatus, endoplasmic reticulum, endosomes, and other membrane compartments, while palmitoylation cycles, prenyl-binding proteins such as PDE6δ, and changes in HVR electrostatics regulate access to specific membrane environments and nanoclusters. The functional state of RAS may therefore reflect the combined effects of nucleotide status, membrane localization, and nanoscale organization.

S181 phosphorylation has been associated with both enhanced oncogenic signaling and suppression of KRAS function. Introduction of a negative charge into the polybasic region weakens electrostatic interactions with acidic phospholipids and reduces plasma membrane nanoclustering, providing a mechanistic basis for attenuation of canonical signaling from the plasma membrane. Loss of plasma membrane association, however, does not necessarily terminate signaling, because relocalized KRAS may engage distinct effectors from intracellular compartments. The apparently opposing effects attributed to S181 phosphorylation may thus depend on cell type, membrane composition, calmodulin availability, KRAS activation state, and downstream signaling context. Although ubiquitination is classically associated with proteasomal degradation, RAS ubiquitination can also alter conformation, trafficking, subcellular localization, and effector interactions. Its consequences therefore depend on the modified residue, ubiquitin linkage type, and cellular compartment. GSK3/β-TrCP-dependent ubiquitination can promote RAS degradation, whereas Rabex-5-dependent ubiquitination suppresses signaling primarily by redistributing RAS away from signaling-competent membrane domains. Thus, in the RAS context, ubiquitination can function as a spatial and signaling regulator as well as a proteolytic signal.

PTM crosstalk adds another level of regulation. Phosphorylation can generate recognition sites for ubiquitination machinery, as illustrated by phosphorylation-dependent recruitment of β-TrCP or Rabex-5. Individual lysine residues may also serve as competing substrates for different modifications. Lys104 and Lys147, for example, can undergo both ubiquitination and acetylation, raising the possibility that these modifications form mutually exclusive switches at the same residue. PTMs can also occur sequentially: SETD7-mediated methylation of KRAS Lys182/Lys184 has been linked to subsequent RABGEF1-mediated K48-linked polyubiquitination. Such observations have prompted the concept of a RAS “PTM code,” in which the consequence of a given modification depends in part on the modification state of the surrounding protein. Cellular and tissue context can further modify the consequences of individual PTMs. The abundance of kinases, phosphatases, ubiquitin ligases, deubiquitinases, trafficking factors, and membrane lipids varies across tissues and tumor types, such that the same modification may produce different, or even opposing, phenotypes in different cellular environments. This context dependence also complicates therapeutic manipulation of RAS PTMs.

Methodological limitations are also likely to contribute to inconsistent conclusions across studies. Conventional immunoblotting and PTM-specific antibodies can establish the presence of a modification but provide limited information about absolute stoichiometry, linkage type, spatial distribution, or temporal dynamics. Quantitative mass spectrometry, DiGly enrichment, AQUA-based measurements, linkage-specific ubiquitin detection, real-time biosensors, and emerging nanopore-based approaches can provide greater resolution of these features. Combining such methods with endogenous genetic perturbation is particularly important for distinguishing correlation from causation. CRISPR-based site-specific editing can reduce artifacts associated with overexpression and conventional phosphomimetic or modification-deficient mutants, although amino acid substitution itself does not necessarily reproduce the biochemical properties of the corresponding PTM state. This limitation is particularly relevant to lysine methylation, which does not alter side-chain charge in the same way as acetylation. Lys-to-Arg or Lys-to-Gln substitutions therefore cannot faithfully reproduce methylated or unmethylated states. More direct chemical biology approaches, including genetic code expansion and

site-specific installation of defined PTMs, will be needed to determine the structural and functional consequences of these modifications.

The field is increasingly moving beyond a linear "one PTM-one function" model toward a framework in which multiple modifications, membrane compartments, and RAS isoforms jointly shape signaling output. Within this framework, PTMs may regulate not only the overall level of RAS activity but also which fraction of RAS is signaling competent, where signaling occurs, and which effectors are engaged. Key unresolved questions include the endogenous stoichiometry and subcellular distribution of individual PTMs, the temporal order and competition among modifications, their isoform- and tissue-specific regulatory logic, and their causal contributions to tumor phenotypes. Integrating quantitative proteomics, spatial imaging, structural biology, and endogenous genetic approaches could ultimately enable a more complete spatiotemporal map of RAS PTMs.

Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used generative AI tools only to improve language and readability. Specifically, the authors used ChatGPT, Grammarly, *etc.* for language polishing only/for drafting/for rewriting/for idea development in limited sections/substantial portions/entire manuscript. The authors take full responsibility for the content of the manuscript.

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Authors' contribution

Conceptualization, X.L.; literature collection, N.Y., L.Y. and R.Z.; investigation, N.Y., L.Y. and X.L.; supervision, X.L.; resources, R.Z. and J.W.; project administration, X.L.; funding acquisition, X.L.; writing—original draft preparation, N.Y.; writing—review and editing, N.Y., X.L. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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