IJHSR
ijhighschoolresearch.org
REVIEW ARTICLE

Targeting the Undruggable: Small Molecule Modulation of Ras Proteins

Juhan Kim

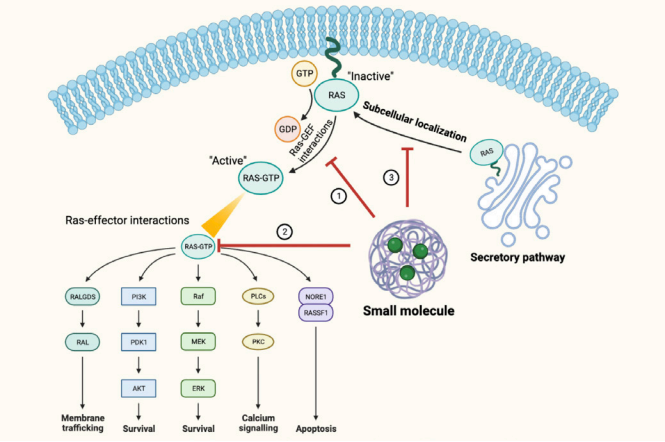
St. Mark's School, 25 Marlboro Rd, Southborough, MA, 01772, U.S.A; rkim7527@gmail.com

ABSTRACT: Ras proteins control critical signaling pathways that regulate cell growth and proliferation. Mutations in Ras are responsible for 30% of all human cancers, making them important targets for therapeutics. Despite enormous efforts in developing therapeutic agents against Ras, minimal success has been observed in clinical settings. Moreover, mutated Ras isoforms have been shown to be unresponsive to established therapies, leading to the notion that Ras may be "undruggable." This review examines several recent studies that utilized methods in chemical biology to target Ras signaling.

KEYWORDS: Chemistry, Other, Chemical Biology, Cancer, Ras Proteins.

Introduction

In our bodies, cell proliferation only happens under specific circumstances, which is possible due to the tight regulation by a network of complex signaling pathways. However, when there is a mutation in these signaling pathways, cells can grow in an uncontrolled manner, leading to cancer. The mutation usually involves the malfunctioning of proteins that possess the ability to regulate cell growth, widely known as proto-oncogenes. Among proto-oncogenes, Ras proteins have attracted much attention because they play essential roles in modulating the activity of many major signaling pathways required for normal cellular proliferation.[1] During signal transduction, Ras proteins switch between "off" and "on" states upon their binding to GTP, which is regulated by guanine nucleotide exchange factors (GEFs).[2] The binding of GTP induces conformational changes and activates Ras proteins, enabling them to interact with downstream effectors, such as PI3K and Raf (Scheme 1).[1] Ras signaling is heavily dependent on the correct cellular localization of Ras, in which the protein must be positioned in the plasma membrane.[3] The intracellular trafficking of Ras is determined by post-translational modifications, such as lipidation and interaction with solubilizing protein factors.[2] Specific mutations in the Ras gene induce the constitutive activation of Ras, with K-Ras being the most frequently mutated isoform.[2] Ras mutants are "stuck" in their active state, causing cells to grow uncontrollably and become unresponsive to apoptosis signals. Mutated Ras is perhaps one of the most significant drivers of cancer, as they are associated with 30% of all human cancers.



Scheme 1: Recent approaches in using small molecules to modulate Ras-signaling. The three main branches are: preventing the formation of the Ras-GTP complex by interfering with Ras-GEF interaction (shown as 1), developing inhibitors of Ras-effector interactions (shown as 2), and suppressing the subcellular localization of Ras proteins (shown as 3). These approaches ultimately aim to inhibit the activity of mutated Ras, preventing the uncontrolled growth of tumor cells.

Over the past 30 years, researchers have made great strides toward understanding the Ras signaling pathway at the molecular level. Accompanied by this progress, many scientists have attempted to target mutated Ras proteins for cancer therapy. However, no study has succeeded in clinical trials, causing the scientific community to consider Ras "undruggable."[3] Two factors that make Ras extremely difficult to target. First, these mutants have a picomolar affinity for GTP and thus are often frozen in their "on" state.[1] Second, Ras proteins lack suitable surfaces for small molecules to bind. Besides the nucleotide-binding pocket, these proteins do not possess any additional binding domains.[3] Even with in-depth structural analysis and iterative sounds of synthetic screening, scientists were not able to identify a feasible binding site on Ras proteins.[2] In addition, multiple studies have reported that tumors can quickly adapt to Ras inhibitors by inducing the lipidation

DOI: 10.36838/v7i9.103

of H-Ras and K-Ras isoforms, further hampering the success of Ras-targeted therapies.[5]

The status quo on Ras-targeted therapies has called scientists to develop new approaches. Recent advances in science, especially in the field of chemical biology, have provided scientists with powerful tools to combat this challenging problem. Recent attempts can be widely characterized into three groups: 1) inhibiting the formation of Ras-GTP complex, 2) blocking Ras-effector interactions, and 3) suppressing the cellular localization of Ras (Scheme 1). This paper will cover some of the illustrative examples of each approach and evaluate whether these endeavors offer hope in targeting the "undruggable."

Discussion

Approach I: Preventing the Formation of Ras-GTP:

Inspired by ATP-competitive kinase inhibitors, early studies attempted to develop Ras inhibitors by directly competing against binding with GTP molecules.[6,7] However, these inhibitors exhibited micromolar affinity toward Ras and could not out-compete the picomolar affinity between Ras-GTP.[8] Thus, instead of competing directly against the nucleotide, scientists turned to investigate strategies that prevent the initial formation of the Ras-GTP complex. Peri and Patgiri aimed to accomplish this task by inhibiting the GEF-catalyzed nucleotide exchange reaction.[9,10] Due to the high affinity of Ras to GDP, the conversion of GDP to GTP needs to be catalyzed by nearby GEFs, such as RasGRF1 and SOS.[3] During the RasGRF1-catalyzed reaction, the catalytic domain of RasGRF1 interacts with the switch I and switch II domains of Ras to open up the nucleotide-binding site,[11] allowing for the release of GDP (Figure 1a). Using virtual ligand docking methods,[12] Peri identified a series of bicyclic scaffolds derived from the natural sugar D-arabinose that bind to the Ras switch II region (Figure 1b). Molecular modeling results showed that the aromatic residues of these sugar-derived molecules form π-stacking interactions with the phenylhydroxylamine groups near the switch II region, forming a stable product.[9] The binding of small molecules to the Ras switch II domain disrupts the Ras-RasGRF1 interaction, thus preventing the conversion of GDP to GTP. When these Ras inhibitors were characterized in vitro, the nucleotide-dissociation assay demonstrated that the sugar derivatives inhibited the release of GDP in a concentration-dependent manner.[9] Notably, one of the lead compounds showed a similar nucleotide dissociation rate compared to that of intrinsic GTPase activity.[9]

Similarly, Patgiri developed an orthosteric inhibitor of Ras, but instead aimed to suppress the SOS-catalyzed nucleotide exchange reaction.[10] During the SOS-catalyzed reaction, the SOS helical hairpin domain is inserted into the switch regions of Ras, disrupting water-mediated intermolecular interactions between Ras and guanine nucleotide, ultimately destabilizing the GDP-bound state of Ras.[11] Based on structural and biochemical analyses of Ras-SOS interactions, researchers identified that F929 and N944 contribute most strongly to the binding of the hairpin domain to Ras.[11] Then, Patgiri utilized the hydrogen bond surrogate (HBS) approach[13] to design synthetic α-helix mimics that stabilize the GDP-bound state of Ras, preventing the release of GDP (Figure 1c). In *in vitro* assays, these α-helical peptides significantly suppressed nucleotide exchange as compared to the negative control, illustrating that they can act as an orthosteric inhibitor of Ras-SOS interactions.[10]

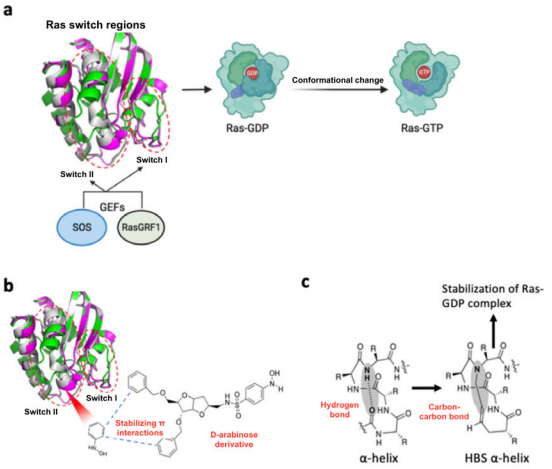


Figure 1: a) Conformation of Ras proteins showing the switch I and switch II regions (indicated with red dotted lines). Guanine nucleotide exchange factors (GEFs) catalyze the conversion of GDP to GTP by interacting with specific domains of the switch I and II regions, ultimately opening the nucleotide-binding site of Ras. (b) Inhibition of RasGRF1-catalyzed nucleotide exchange reaction by D-arabinose-derived bicyclic molecules. The two aromatic residues form stabilizing interactions with the phenylhydroxylamine group of the switch II region, disrupting Ras-RasGRF1 interaction. (c) Structural differences between a regular α-helix and a hydrogen bond surrogate (HBS) α-helix. The HBS strategy affords preorganized α-helices in which the N-terminal main chain hydrogen bond between the C=O of the "ith" amino acid residue and the NH of the "i+4th" amino acid residue is replaced with a carbon-carbon bond (shown by grey circles). HBS α-helix stabilizes the Ras-GDP complex, disrupting Ras-SOS interaction.

Both studies successfully identified small-molecule inhibitors that inhibit Ras-dependent cell proliferation at high micromolar concentrations. Orthosteric inhibitors of Ras-RasGRF1 and Ras-SOS interactions effectively downregulated cell growth in p21 human Ras and HeLa cells, respectively.[9,10] However, sugar derivatives are known to be unstable in organic solvents and water at room temperature.[14] Furthermore, Ras-RasGRF1 inhibitors showed poor results in vivo and even displayed toxic effects.[9] In the case of α-helix mimics, they had a tenfold lower affinity for Ras-GDP than the parent SOS itself, which would lead to limited therapeutic effects. Moreover, the exact mode of action remains elusive, hindering further optimization.[10] Most importantly, a fundamental limitation of these Ras-GEF inhibitors is that they do not discriminate between mutated and wild-type Ras proteins. Without targeting cancer-targeting moieties, the inhibitors will prevent the formation of the Ras-GTP complex in healthy cells, which can lead to undesirable outcomes, including cell death.[3]

Approach II: Inhibiting Ras-effector Interactions:

An alternative approach in Ras inhibition is to suppress the interactions between Ras and its downstream effectors, which regulate various signaling pathways (Scheme 1). When Ras proteins interact with their effectors, they readily transition between two distinct states, in which state 1 represents

a conformation with a reduced affinity for effector binding (Figure 2a). The affinity of state 1 for effectors is reported to be 20 times weaker.[15] Notably, multiple studies have discovered that the weak binding state possesses potential binding sites for small molecules on its surface.[15,16] Based on this finding, Rosnizeck developed allosteric inhibitors of Ras based on organometallic motifs.[17] Their goal was to stabilize the weak binding state to discourage its interaction with effector molecules. Through 31P NMR, the researchers identified that the zinc (II) complex of 1,4,7,10-tetraazacyclododecane complex ($Zn2^+$ cyclen) selectively binds to the surface of the weak-affinity state.[17,18] Importantly, the organometallic molecule significantly stabilized the weak affinity state, thus shifting the equilibrium toward state 1 and discouraging the formation of the strong affinity state (Figure 2b). However, $Zn2^+$ cyclen only showed millimolar affinity toward Ras, and the downregulation of Ras-dependent pathways was not observed in animal studies.[17] Moreover, organometallic compounds suffer from unfavorable pharmacological properties, including but not limited to formulation barriers, off-target issues, systemic toxicity, and engagement in redox reactions.[19] These limitations raise a critical concern for the use of organometallic inhibitors *in vivo*.

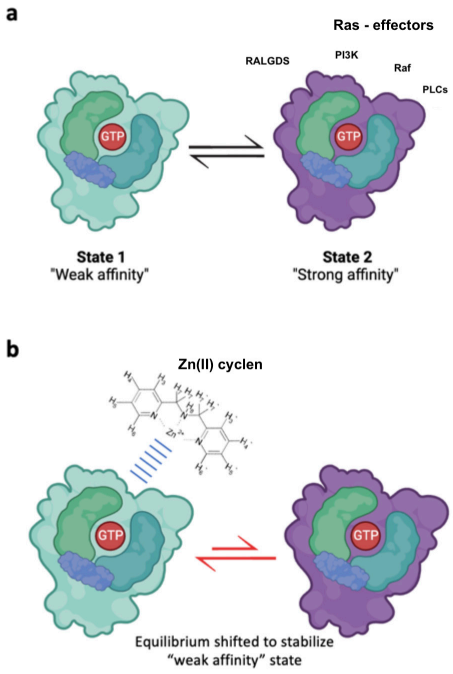


Figure 2: (a) Dynamic equilibrium between the weak-affinity (state 1) and strong affinity conformations (state 2). Ras-GTP complexes constantly switch between the two conformations. Effector molecules of Ras bind to the Ras-GTP complex when the complex is in state 2. (b) Allosteric inhibition of Ras-effector interactions using organometallic compounds. Zn^{2+}cyclen selectively binds and stabilizes the weak-affinity state. As a result, the equilibrium is shifted toward state 1, making the transition to state 2 thermodynamically unfavorable.

To overcome the unfavorable properties of organometallic inhibitors, Tanaka and Rabbitts explored different methods to inhibit Ras-effector interactions. In particular, they were interested in finding antibodies that bind to the Ras-GTP complex. The researchers screened a library of antibodies in yeast with an H-Ras mutant and isolated a single immunoglobulin heavy chain variable domain fragment (named iDab#6) that selectively binds to the weak affinity state of Ras-GTP (Figure 3a).20 When the binding of iDab#6 to Ras was characterized using luciferase assays, the antibody fragment was specifically bound to positions 12 and 61 of the switch I region of the Ras-GTP complex.[21] This was an important finding because major Ras-effectors, such as PI3K and Raf, bind to the switch I region of Ras,[22] meaning that iDab#6 can act as a competitive inhibitor of Ras-effector interactions. Furthermore, Tanaka and Rabbitts observed that iDab#6 interacts with mutant H-Ras with at least 10 times higher binding than with wild-type H-Ras, allowing the specific targeting of mutant Ras.[20] To test whether the antibody fragment can downregulate Ras-dependent signaling pathways, iDab#6 was introduced to mouse tumor models bearing lung cancer cells. Based on the results, the introduction of iDab#6 led to an increased survival rate and reduced tumor size, demonstrating the antibody's ability to suppress Ras-effector interactions.[21]

Other scientists aimed to discover peptide-based orthosteric inhibitors of Ras-effector interactions. In a recent study, Wu and Upadhyaya utilized a combinatorial screening method to identify a cyclic peptide that selectively binds to the weak-affinity state of Ras-GTP.[23] A library of 6×10^6 cyclic peptides was synthesized, in which each bead contained a random peptide sequence of four to six amino acid residues on its surface (Figure 3b). Each position was randomized with a 25-amino acid set to maximize structural diversity and protease resistance.[23] To further increase diversity, the researchers varied the ring size at each position by cyclizing an aliquot of the library peptides. Next, the library was screened against the G12V K-Ras, one of the most frequently observed K-Ras mutants.[3] Using this method, researchers were able to produce more than six million unique compounds and screened each of them, which speaks to the power of combinatorial science. Screening of the library produced around 20 lead compounds that selectively bind to state 1 of the Ras-GTP complex.[23] Interestingly, the lead compounds were dominated by larger rings and rich in aromatic residues. The exact reason behind this finding is unclear; however, large rings may form favorable interactions with aromatic residues of the switch I domain, establishing a steric block between Ras and its effectors. Homogenous time-resolved fluorescence (HTRS)[24] indicated that the cyclic peptides inhibit the Ras-Raf interaction with micromolar affinity, leading to the downregulation of the MAPK pathway (Scheme 1). In cellular assays, the small molecule inhibitors also led to a decreased phosphorylation of Mek and Erk, two downstream effectors of Ras, that promote tumor survival when phosphorylated (Scheme 1).

Even though orthosteric inhibitors developed by Wu and Tanaka displayed promising in vivo and in vitro results, there are several limitations. First, the size of these small molecules poses a problem, especially in the case of cyclic peptide inhibitors. Findings revealed that incorporating large aromatic residues favors binding with Ras,[23] meaning that the size of the resulting inhibitor will be relatively large. Size is a critical

DOI: 10.36838/v7i9.103

factor in therapeutic efficacy, and the large size of the peptide will likely pose problems when crossing the plasma membrane. Second, these studies targeted specific isoforms of Ras mutants. However, there are multiple Ras isoforms, and a single isoform is not responsible for driving tumor progression.1 There is no guarantee that an inhibitor that downregulates K-Ras will work for H-Ras or N-Ras. The genetic makeup of Ras isoforms will vary depending on the type of cancer and individual patients, which complicates the use of these inhibitors in clinical studies. Most importantly, no molecular-level evidence exists of the interaction between the small molecule inhibitors and the Ras-GTP complex. This is a crucial limitation that prevents further development of these Ras inhibitors.

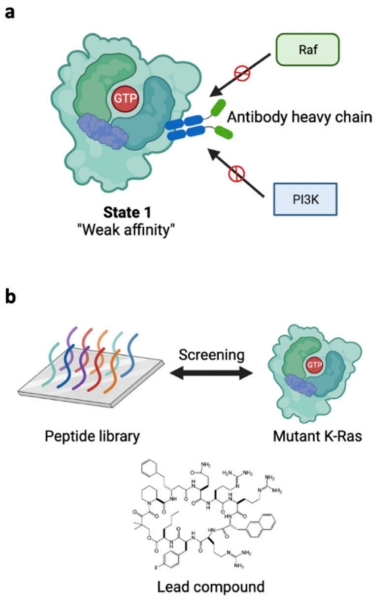


Figure 3: (a) Orthosteric inhibition of Ras-effector interactions by iDab#6. The antibody fragment selectively binds to the switch I domain of mutant H-Ras, a binding site of major Ras-effector molecules. The administration of IDab#6 into mouse xenograft models significantly reduced tumor growth and proliferation. (b) Synthesis of cyclic peptide inhibitors using combinatorial science. The library included about six million peptides, screened against mutant K-Ras molecules. The lead compound containing large aromatic residues led to notable downregulation of Ras-Raf interactions and inhibited tumor proliferation.

Approach III: Impairing Ras Subcellular Localization:

The final approach in small molecule modulation of Ras signaling is through impairing its intracellular localization. Ras-dependent signaling pathways that promote tumor cell survival and metastasis depend on the correct localization of Ras at the plasma membrane, 4 which is enabled by a series of post-translational processing reactions (Figure 4a). These modifications include cysteine S-farnesylation, cysteine S-palmitoylation, proteolysis, and C-terminal carboxymethylation.[25] Among them, the addition of farnesyl groups to the CAAX cysteine thiol by farnesyltransferase (FTase) has been reported as a major driver of Ras cellular localization, as highlighted by the inability of Ras mutants lacking the C-terminal cysteine to localize to the plasma membrane.[4,25,26] This observation prompted the development of farnesyltransferase inhibitors (FTIs) as potential anticancer drugs. Kohl and Mosser were among the first groups of scientists to design FTIs by designing CAAX motif derivatives.27 When a panel of tetrapeptide analogs of the CAAX motif was screened, N-2(S)-[2(R)-amino-3-mercaptopropylamino]3(S)-methylpentyl isoleucyl-homoserine lactone showed selective binding to FTase (Figure 4b). The N-terminal peptide bonds were reduced for resistance against hydrolysis by mammalian aminopeptidases, while the C-terminal serine was cyclized to facilitate membrane penetration by masking the anionic carboxylate.[28] In cellular assays, the tetrapeptide selectively inhibited FTase by out-competing the substrate farnesyl diphosphate, leading to an increased concentration of inactive, cytosolic Ras proteins. Its potency was also demonstrated in mouse models, in which the injection of the CAAX mimetic led to a significant decrease in tumor growth and proliferation.[27] Since Kohl and Mosser's work, there have been many efforts in optimizing the CAAX motif-based inhibition and elucidating its molecular mechanism.[4, 26,29]

Instead of competing directly with the substrate farnesyl diphosphate, some studies sought methods to compete against Ras proteins for binding with farnesyl-binding proteins within the plasma membrane. Campbell and Boufaied utilized DECIPHER technology,[30] a genomics and bioinformatics platform that predicts the structures of secondary metabolites based on bacterial genomic sequences, to discover a farnesyl cysteine mimetic.[31] Their lead compound, TLN-4601, is a farnesylated dibenzodiazepinone that selectively binds to the peripheral benzodiazepine receptor, which shares many structural similarities to farnesyl-binding proteins (Figure 4c).[32] Campbell and Boufaied hypothesized that TLN-4601 would interfere with Ras localization by binding to farnesyl-binding proteins. To evaluate whether the farnesyl mimetic interferes with Ras-dependent signaling, the researchers treated human pancreatic epithelial cells with 10 μM of TLN-4601.[31] As expected, TLN-4601 treatment resulted in decreased phosphorylation of Raf-1, MEK, and ERK1/2, all of which are downstream effectors of Ras that regulate tumor cell survival (Scheme 1).[31] Furthermore, promising anti-tumor effects were observed in mouse xenograft models, suggesting that TLN-4601 is a good candidate for future clinical studies.

Despite promising preclinical efficacy, FTIs and farnesyl mimetics were ineffective in clinical trials, and data suggest that only a small subset of patients respond to these Ras inhibitors.[33] The major reason for the discrepancy between laboratory findings and clinical data is the high mutation rate of K-Ras and N-Ras isoforms. Mutant Ras proteins can be alternatively prenylated by gamma-glutamyltransferase 1, obviating their dependence on farnesylation for their correct subcellular localization.[26] Recent findings suggest that there may be more enzymes that lapidate mutant Ras.[34] Moreover, these mutant Ras are fully functional and can bind to a series of lipid-binding proteins, significantly hampering the efficacy of farnesyl mimetics.[29] From this point, a major effort must be put into identifying tumors that depend on farnesylation for proliferation and characterizing the human prenylome to increase therapeutic efficacy in clinical trials.

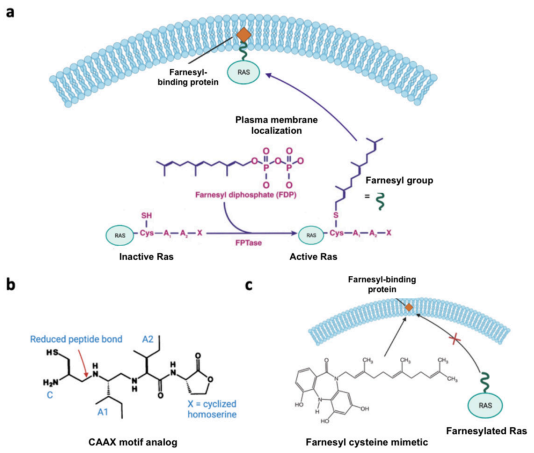


Figure 4: (a) Subcellular localization of Ras proteins· Ras is synthesized as a cytosolic precursor that ultimately localizes to the cytoplasmic face of the plasma membrane. Farnesyl transferase covalently adds farnesyl diphosphate to the C-terminal cysteine of inactive Ras. Farnesylated Ras is then localized to the plasma membrane by binding to farnesyl-binding proteins. (b) Structure of the CAAX motif analog, N-2(S)-[2(R)-amino-3-mercaptopropylamino]3(S)-methylpentyl isoleucyl-homoserine lactone. (c) TLN-4601 competes with farnesylated Ras in binding to farnesyl-binding proteins.

Conclusion

To sum up, the recent developments in the field of chemical biology have created segways to use small molecules to prevent Ras-signaling. Aberrant Ras signaling is a crucial problem to be solved, as it is one of the biggest drivers of cancer in humans, and most importantly, there is no method to control Ras signaling now. Small molecules can be used in three aspects: 1) preventing the formation of Ras-GTP, downregulating interactions between Ras and its effector molecules, and 3) stopping the subcellular localization of Ras onto plasma membranes. However, despite these promising approaches, the complete inhibition of Ras-signaling remains a challenge. Limited success in clinical trials raises the question of whether a single approach or a specific type of small molecule can downregulate Ras-signaling. In addition, multiple studies have reported how Ras-dependent pathways are upregulated in response to drug treatments.[34] Such a response ultimately leads to treatment evasion and increases the dose, causing pharmacokinetic complications.[35] Therefore, future research should aim to decipher the adaptive resistance mechanisms of cancer cells in response to small-molecule treatments. Moreover, structural investigation on Ras isoforms and interactions with their effector molecules is required, which can reveal novel sites for small molecule interventions and ways to develop personalized treatment strategies.[34] To translate these molecular findings into effective treatments, it will be crucial to bridge chemical biology with systems biology, AI-driven screening, computational biology, and clinical oncology.[36]

On the positive side, advances in combinatorial science and bioinformatics have allowed researchers to make further leaps in discovering novel inhibitors of Ras. For example, scientists have recently discovered small-molecule binding pockets in the switch regions of Ras.[37,38] Researchers have also shown that integrating nanotechnology with existing therapies can significantly enhance pharmacokinetics and drug delivery.[39,40] Noting that Ras is one of the major drivers of human cancer pathogenesis, continuous efforts should be made to develop multidisciplinary approaches to inhibit aberrant Ras-signaling. Although Ras was widely considered "undruggable" over the past decades, recent findings discussed above have revived the hope that Ras is "yet to be drugged." As chemical biology and related fields continue to advance, the likelihood of effective therapeutic intervention of Ras-signaling steadily increases.

Acknowledgments

I would like to express my deepest gratitude to Dae Kyu Kim, whose guidance and mentorship have been invaluable throughout the project. Dae Kyu not only guided the direction of my research but also encouraged me to critically analyze scientific perspectives.

References

1. Wennerberg, K.; Rossman, K. L.; Der, C. J. The Ras superfamily at a glance. *Journal of Cell Science* **2005**, *118* (5), 843-846.
2. Chen, K.; Zhang, Y.; Qian, L.; Wang, P. Emerging strategies to target RAS signaling in human cancer therapy. *Journal of hematology & oncology* **2021**, *14*, 1-23.
3. Perurena, N.; Situ, L.; Cichowski, K. Combinatorial Strategies to Target RAS-Driven Cancers. *Nat. Rev. Cancer* **2024**, *24* (5), 316–337.
4. Frech, M.; John, J.; Pizon, V.; Chardin, P.; Tavitian, A.; Clark, R.; McCormick, F.; Wittinghofer, A. Inhibition of GTPase activating protein stimulation of Ras-p21 GTPase by the K rev-1 gene product. *Science* **1990**, *249* (4965), 169-171.
5. Ramos-Kuri, M.; *et al*. Molecules Linked to Ras Signaling as Therapeutic Targets in Cardiac Pathologies. *Biol. Res.* **2021**, *54* (1), 23.
6. Becher, I.; Savitski, M. M.; Savitski, M. F. l.; Hopf, C.; Bantscheff, M.; Drewes, G. Affinity profiling of the cellular kinome for the nucleotide cofactors ATP, ADP, and GTP. *ACS Chemical Biology* **2013**, *8* (3), 599-607.
7. Peri, F.; Airoldi, C.; Colombo, S.; Martegani, E.; van Neuren, A. S.; Stein, M.; Marinzi, C.; Nicotra, F. Design, synthesis and biological evaluation of sugar-derived Ras inhibitors. *ChemBioChem* **2005**, *6* (10), 1839-1848.
8. Patgiri, A.; Yadav, K. K.; Arora, P. S.; Bar-Sagi, D. An orthosteric inhibitor of the Ras-Sos interaction. *Nature Chemical Biology* **2011**, *7* (9), 585-587.
9. Cherfils, J.; Zeghouf, M. Regulation of small gtpases by gefs, gaps, and gdis. *Physiological Reviews* **2013**, *93* (1), 269-309.
10. Taveras, A. G.; Remiszewski, S. W.; Doll, R. J.; Cesarz, D.; Huang, E. C.; Kirschmeier, P.; Pramanik, B. N.; Snow, M. E.; Wang,Y. S.; del Rosario, J. D.; *et al*. Ras oncoprotein inhibitors: the discovery of potent, ras nucleotide exchange inhibitors and the structural determination of a drug-protein complex. *Bioorg Med Chem* **1997**, *5* (1), 125-133. DOI: 10.1016/s0968-0896(96)00202-7 From NLM.
11. Patgiri, A.; Jochim, A. L.; Arora, P. S. A hydrogen bond surrogate approach for stabilization of short peptide sequences in alpha-helical conformation. *Acc Chem Res* **2008**, *41* (10), 1289-1300. DOI: 10.1021/ar700264k From NLM.
12. Kazanis, S.; McClelland, R. A. Electrophilic intermediate in the reaction of glutathione and nitroso arenes. *Journal of the American Chemical Society* **1992**, *114* (8), 3052-3059. DOI: 10.1021/ja00034a043.
13. Geyer, M.; Schweins, T.; Herrmann, C.; Prisner, T.; Wittinghofer, A.; Kalbitzer, H. R. Conformational transitions in p21 ras and in its complexes with the effector protein Raf-RBD and the GTPase activating protein GAP. *Biochemistry* **1996**, *35* (32), 10308-10320.

DOI: 10.36838/v7i9.103

14. Spoerner, M.; Herrmann, C.; Vetter, I. R.; Kalbitzer, H. R.; Wittinghofer, A. Dynamic properties of the Ras switch I region and its importance for binding to effectors. *Proceedings of the National Academy of Sciences* **2001**, *98* (9), 4944-4949.
15. Rosnizeck, I. C.; Graf, T.; Spoerner, M.; Tränkle, J.; Filchtinski, D.; Herrmann, C.; Gremer, L.; Vetter, I. R.; Wittinghofer, A.; König,
16. B. Stabilizing a weak binding state for effectors in the human ras protein by cyclen complexes. *Angewandte Chemie International Edition* **2010,** *49* (22), 3830-3833.
17. Kalbitzer, H.; Spoerner, M. State 1(T) Inhibitors of Activated Ras. Vol. 33; 2013; pp 69-95.
18. Martins, P.; Marques, M.; Coito, L.; Pombeiro, A. J.; Baptista, P. V.; Fernandes, A. R. Organometallic compounds in cancer therapy: past lessons and future directions. *Anticancer Agents Med Chem* **2014**,*14* (9), 1199-1212. DOI: 10.2174/18715206146661408291 2 4925 From NLM.
19. Tanaka, T.; Williams, R. L.; Rabbitts, T. H. Tumour prevention by a single antibody domain targeting the interaction of signal transduction proteins with RAS. *Embo J* **2007**, *26* (13), 3250-3259. DOI: 10.1038/sj.emboj.7601744 From NLM.
20. Tanaka, T.; Rabbitts, T. Interfering with RAS–effector protein interactions prevent RAS-dependent tumour initiation and causes stop–start control of cancer growth. Oncogene 2010, 29 (45), 6064-6070. Vetter, I. R.; Wittinghofer, A. The guanine nucleotide-binding switch in three dimensions. *Science* **2001**, *294* (5545), 1299-1304. DOI: 10.1126/science.1062023 From NLM.
21. Wu, X.; Upadhyaya, P.; Villalona-Calero, M. A.; Briesewitz, R.; Pei, D. Inhibition of Ras–effector interactions by cyclic peptides. *MedChemComm* **2013**, *4* (2), 378-382.
22. Leyris, J. P.; Roux, T.; Trinquet, E.; Verdié, P.; Fehrentz, J.-A.; Oueslati, N.; Douzon, S.; Bourrier, E.; Lamarque, L.; Gagne, D.; et al. Homogeneous time-resolved fluorescence-based assay to screen for ligands targeting the growth hormone secretagogue receptor type 1a. *Analytical Biochemistry* **2011**, *408* (2), 253-262.
23. Ahearn, I. M.; Haigis, K.; Bar-Sagi, D.; Philips, M. R. Regulating the regulator: post-translational modification of RAS. *Nature Reviews Molecular Cell Biology* **2012**, *13* (1), 39-51.
24. Berndt, N.; Hamilton, A. D.; Sebti, S. M. Targeting protein prenylation for cancer therapy. *Nature Reviews Cancer* **2011**, *11* (11), 775-791.
25. Kohl, N. E.; Mosser, S. D.; deSolms, S. J.; Giuliani, E. A.; Pompliano, D. L.; Graham, S. L.; Smith, R. L.; Scolnick, E. M.; Oliff, A.; Gibbs, J. B. Selective inhibition of ras-dependent transformation by a farnesyltransferase inhibitor. *Science* **1993**, *260* (5116), 1934-1937. DOI: 10.1126/science.8316833 From NLM.
26. Orgován, Z.; Keserű, G. M. Small molecule inhibitors of RAS proteins with oncogenic mutations. *Cancer and Metastasis Reviews* **2020**, *39* (4), 1107-1126.
27. Prior, I. A.; Lewis, P. D.; Mattos, C. A comprehensive survey of Ras mutations in cancer. *Cancer Res* **2012**, *72* (10), 2457-2467. DOI: 10.1158/0008-5472.Can-11-2612 From NLM.
28. McAlpine, J. B.; Bachmann, B. O.; Piraee, M.; Tremblay, S.; Alarco, A.-M.; Zazopoulos, E.; Farnet, C. M. Microbial genomics as a guide to drug discovery and structural elucidation: ECO-02301, a novel antifungal agent, as an example. *Journal of Natural Products* **2005**, *68* (4), 493-496.
29. Campbell, P. M.; Boufaied, N.; Fiordalisi, J. J.; Cox, A. D.; Falardeau, P.; Der, C. J.; Gourdeau, H. TLN-4601 suppresses growth and induces apoptosis of pancreatic carcinoma cells through inhibition of Ras-ERK MAPK signaling. *Journal of Molecular Signaling* **2010**, *5* (1), 1-10.
30. Boufaied, N.; Wioland, M. A.; Falardeau, P.; Gourdeau, H. TLN-4601, a novel anticancer agent, inhibits Ras signaling post Ras prenylation and before MEK activation. *Anticancer Drugs* **2010**, *21* (5), 543-552. DOI: 10.1097/CAD.0b013e328337f373 From NLM.
31. Rowell, C. A.; Kowalczyk, J. J.; Lewis, M. D.; Garcia, A. M. Direct demonstration of geranylgeranylation and farnesylation of K-Ras *in vivo*. *Journal of Biological Chemistry* **1997**, *272* (22), 14093-14097.
32. Stephen, A. G.; Esposito, D.; Bagni, R. K.; McCormick, F. Dragging back in the ring. *Cancer Cell* **2014,** *25* (3), 272-281.
33. Prahallad, A.; Sun, C.; Huang, S.; Di Nicolantonio, F.; Salazar, R.; Zecchin, D.; Beijersbergen, R. L.; Bardelli, A.; Bernards, R. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* **2012**, *483* (7387), 100-103. DOI: 10.1038/nature10868.
34. Maurer, T.; Garrenton, L. S.; Oh, A.; Pitts, K.; Anderson, D. J.; Skelton, N. J.; Fauber, B. P.; Pan, B.; Malek, S.; Stokoe, D. Small-molecule ligands bind to a distinct pocket in Ras and inhibit SOS-mediated nucleotide exchange activity. *Proceedings of the National Academy of Sciences* **2012**, *109* (14), 5299-5304.
35. Grant, B. J.; Lukman, S.; Hocker, H. J.; Sayyah, J.; Brown, J. H.; McCammon, J. A.; Gorfe, A. A. Novel allosteric sites on Ras for lead generation. *PloS one* **2011**, *6* (10), e25711.
36. Doss, C. G. P.; Debottam, S.; Debajyoti, C. Glutathione-responsive nano-transporter-mediated siRNA delivery: silencing the mRNA expression of Ras. *Protoplasma* **2013**, *250*, 787-792.
37. Aslan, B.; Ozpolat, B.; Sood, A. K.; Lopez-Berestein, G. Nanotechnology in cancer therapy. *Journal of Drug Targeting* **2013**, *21* (10), 904-913.
38. Punekar, S. R.; *et al*. The Current State of the Art and Future Trends in RAS-Targeted Cancer Therapies. *Nat. Rev. Clin. Oncol.* **2022**, *19* (10), 637–655
39. Wei, D.; *et al*. A Small Molecule with Big Impact: MRTX1133 Targets the KRAS^{G12D} Mutation in Pancreatic Cancer. Clin. *Cancer Res.* **2024**, *30* (4), 655–662
40. Wu, D.; *et al*. Small Molecules Targeting Protein Protein Interactions for Cancer Therapy. *Acta Pharm. Sin. B* **2023**, *13* (10), 4060–4088.

Author

Juhan Kim is a high school student in Massachusetts with an interest in the application of chemical tools to alter proteins' biological functions.