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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Elucidating Structural Details of Ras-Effector Interactions

Ras-Efektör Etkileşimlerinin Yapısal Detaylarının Açığa Çıkarılması

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Abstract

Small membrane-associated Ras proteins mediate a wide range of cellular functions, such as cell proliferation, migration, survival, and differentiation; through binding and activating numerous effectors. Constitutively active mutant Ras proteins are detected in various types of human cancer and Ras community seeks approaches other than small-molecule Ras inhibitors; such as targeting the protein-protein interactions in the downstream Ras effector pathways and preventing its membrane localization. Although the most studied effectors of Ras, i.e. Raf, PI3K and RalGDS, bind Ras through the same site, they elicit opposing signaling pathways and thus, the temporal and spatial decision of the cell among them is critical. Elucidating the structural details of Ras-effector interactions can help us understand the cell decision and target the protein-protein interactions precisely. However, only a few crystal structures of Ras in complex with an effector are deposited in PDB. Here, the 3D structures of Ras/effector complexes were modeled with the PRISM algorithm and important binding sites as well as hot spot residues on Ras were identified. The effectors were also classified according to the binding regions on Ras, to determine the competitive pathways and the binding regions other than the "effector lobe". The modeled complexes reveal important information about the interfaces between Ras and its partners with the potential of guiding drug design studies to block oncogenic Ras signaling

Keywords: Ras effectors, oncogenic signaling, protein-protein interaction, protein-protein interface, hot spot, protein structure

Öz

Hücre zarıyla ilintili küçük Ras proteinleri pek çok efektöre bağlanıp onları aktif hale getirerek hücre çoğalması, göçü, hayatta kalma ve farklılaşması gibi çeşitli hücresel işlevleri kontrol ederler. Ras üzerindeki mutasyonlar, yapısal olarak aktif proteine sebebiyet verir ve insandaki birçok kanser tipinde tespit edilmişlerdir ve Ras topluluğu Ras'ı hedef alan küçük moleküllü inhibitörler tasarlamak yerine Ras'ın efektör yolaklarındaki protein-protein etkileşimlerini hedef alarak Ras'ın zar üzerindeki lokalizasyonunu engellemeyi amaçlamaktadır. Ras'ın en çok çalışılan efektörleri, Raf, PI3K ve RalGDS, Ras'a aynı yüzeyden bağlanmasına rağmen karşıt sinyal yolaklarını ortaya çıkarırlar ve dolayısıyla hücrenin bu yolaklar arasındaki zamansal ve mekansal kararları kritik öneme sahiptir. Ras/efektör etkileşimlerinin yapısal detaylarını açığa çıkarmak, hücrenin karar mekanizmasını anlamamıza ve protein-protein etkileşimlerini hassas olarak hedeflememize yardımcı olabilir. Bununla birlikte, sadece birkaç Ras/efektör kompleksinin kristal yapısı PDB'de bulunmaktadır. Bu çalışmada, Ras/efektör komplekslerinin 3 boyutlu yapıları PRISM algoritması ile modellenmiştir ve Ras üzerindeki sıcak nokta kalıntılarının yanı sıra önemli bağlanma bölgeleri belirlenmiştir. Efektörler ayrıca, rekabetçi yolları ve "efektör lobu" dışındaki bağlayıcı bölgeleri belirlemek için Ras'daki bağlayıcı bölgelere göre sınıflandırılmıştır. Modellenen kompleksler, Ras ve ortakları arasındaki arayüzeyler hakkında onkojenik Ras sinyal iletimini bloke etmek için ilaç tasarım çalışmalarına rehberlik etme potansiyeli olan önemli bilgiler ortaya koymaktadır.

Anahtar Kelimeler: Ras efektörleri, onkojenik sinyal iletimi, protein-protein etkileşimi, protein-protein arayüzeyi, protein yapısı

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I. INTRODUCTION

1.1 Ras Family of Proteins

Ras-family proteins regulate diverse functions in the cell such as proliferation, migration, survival, and differentiation [1-2] and they are small membrane-associated GTPases that can activate multiple signaling pathways. Once getting activated by upstream regulators; they recruit and activate their downstream effectors such as Raf, phospho-inositol 3 kinase (PI3K), and Ral guanine nucleotide dissociation stimulator (RalGDS) [3-5], to the plasma membrane and initiate a signaling cascade leading to several responses within the cell. GTPases switch between GTP-bound active (on) and GDP-bound inactive (off) states. Ras can transmit signals in the GTP-bound active state but when GTP is hydrolyzed to GDP, it switches to an off state unable to relay a signal. The intrinsic replacement of GDP by GTP and hydrolysis of GTP to GDP processes are catalyzed within the cell by upstream regulators of Ras, i.e. guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs), respectively [6-7].

1.2 Structure of Ras

The available crystal structures of H-Ras reveal that the catalytic domain of Ras is composed of six β-strands (β1-β6), 4-to-7 α-helices (number of helices differs in structures) (α1-α7) and around ten connecting loops. The functional regions on Ras are p-loop (residues between 10-17), switch I (between 30-38), and switch II (between 59-76) comprising the active nucleotide and effector binding sites (Figure 1) [8]. One of the dimer interfaces on Ras, which is majorly populated, is β-sheet dimer interface at the effector binding site and it overlaps with the binding surfaces of effectors such as Raf, PI3K and RalGDS [9-10]. The first half of the catalytic domain (residues 1-86, called "effector lobe") is identical in Ras isoforms [11-12]. The rest of the molecule, residues 87-166, called "allosteric lobe", contains a more variable sequence among Ras isoforms. This allosteric site (including the last three/four helices and β5-β6 strands) serves as the second half of the catalytic domain [13] and overlaps with the Ras dimerization through the helical interface [10] (Figure 1).

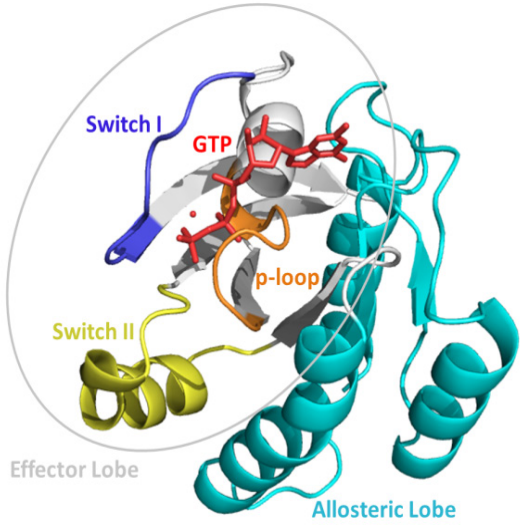

Figure 1. Structure of H-Ras (PDB ID: 1QRA).

1.3 Ras in Cancer

Majority of the oncogenic Ras mutations are at G12, G13 and Q61 residues and they result in impaired GTP hydrolysis, keeping the protein in the constitutively active GTP-bound state. Moreover, these mutant Ras genes are shown to be present in nearly 30% of metastatic cancers [14-15]. Four highly similar isoforms are encoded by human RAS genes: H-Ras, N-Ras, K-Ras4A and 4B [16]. However, the frequency of Ras mutations are not equally distributed between Ras isoforms according to the Catalog of Somatic Mutations in Cancer (COSMIC) database [17]: K-Ras with 86%, N-Ras with 11% and H-Ras with 3% mutation frequency [18]. After more than three decades of research, Ras proteins were classified as "undruggable" [19]. However, allosteric inhibitors that target Ras interactions have recently been reported [20-25] and subsequently, the challenging task of directly targeting Ras has shifted to the leading approach of Ras inactivation through protein-protein interaction disruption [26].

1.4 Effectors of Ras

Once activated, Ras interacts with a wide range of downstream proteins, called "Ras effectors" [27-29], and initiate several signaling pathways in the cell. Although the sequence homology of Ras effectors is low, the Ras Binding Domain (RBD) that binds to active Ras is conserved among the effectors [30]. However, attraction to these domains decreases approximately 1000-fold in the inactive state [31]. The structural details of the interaction between Ras and effector

RBDs are provided by the available crystal structures in PDB [32] (Table 1). The most studied downstream effector of Ras is the serine/threonine kinase Raf, which functions in the Ras-Raf-MEK-ERK signaling cascade [33]. The paralogs of Raf, expressed in mammals are A-, B - and C-Raf (Raf-1). Phosphatidylinositol 3-kinase (PI3K) [34-35] effector of Ras is also important and necessary for activating the Akt family Ser/Thr kinases, which promote apoptosis inhibition and cell survival [36-37]. Another well-known Ras effector is the Ral guanine nucleotide dissociation stimulator (RalGDS) [38-39] functioning in cell vesicular trafficking and NF-κB activation.

Active Ras contributes to the activation of Raf proteins by recruiting them to the plasma membrane [40]. Then, MEK proteins are phosphorylated and activated by Raf, leading to the subsequent phosphorylation and activation of ERK proteins. Ultimately, the propagating signal activates a couple of transcription factors that regulate and promote cell-cycle progression [41]. The C-Raf RBD (residues between 55-131, RafRBD) was shown to directly interact with the H-Ras-GTP [42-44] and the mutations (such as E31, T35, and E37) in switch I loop of H-Ras were shown to significantly impair the association with RafRBD, revealing the importance of the highly conserved switch I region among Ras isoforms for the stability of Ras/RBD of effector complexes [45-46]. Additionally, the crystal structures of wild-type and Q61L mutant H-Ras-GTP (PDB IDs: 4G0N and 4G3X, respectively) in complex with RBD of Raf kinase show that Switch II becomes more rigid upon oncogenic Q61L mutation [47].

II. MATERIALS AND METHODS

2.1 Modeling the 3D structures of Ras complexes with their regulators/effectors and analysis of the interfaces

The effector binding regions on Ras were predicted using PRISM, a protein-protein complex structure prediction algorithm [48-50]. PRISM is a template-based algorithm and the templates used in the predictions are the protein-protein interface motifs that are observed to be conserved and used repeatedly in nature. This prediction algorithm has been tested for different pathways resulting in high accuracy [51]. To build protein-protein interaction complex models, PRISM uses the given target 3D structures of proteins of interest. The resulting models are ranked based on their binding energy scores (BES) computed by FiberDock [52].

Crystal structures of the H-Ras with Raf-1, PI3Kγ, RalGDS, PLCε, Bry22, and RASSF5 are available in PDB (Table 1). Some effectors (RASSF1, RAIN, RGS12, RGL1, AFAD RA2, RIN1) do not have a tertiary structure in PDB, so the 3D models of these proteins were built using I-TASSER [53] (Table 2). G-domains (residues 4-166) of H - and K-Ras were used as targets and their complex structures with the binding partners were modeled. After modeling the complexes, the interface regions of the dimers and energetically important hot spot residues on them were identified using the HotRegion [54]. The results were compared with crystal structures for validation of predictions.

Table 1. Available complexes of human H – and K-Ras with their regulators/effectors in PDB.

Monomer 1	Monomer 2	PDB ID
H-Ras	Sos1	1BKD, 1NVU, 4URU, 4URV, 4URW, 4URX, 4URY, 4URZ, 4US0, 4US1, 4US2,
	p120GAP	1WQ1
	C-Raf	4G0N, 4G3X
	PI3Kgamma	1HE8
	RalGDS	1LFD
	PLCepsilon	2C5L
	Bry2	1K8R
	RASSF5 (Nore1A)	3DDC
K-Ras	Raf	2MSE

Table 2. The list of target protein structures used in PRISM calculations.

Protein	Domain	PDB ID
CDC42	Full-length	2NGR
Sos1	Catalytic domain (GEF fragment)	1BKD
p120GAP	Catalytic domain (GAP fragment)	1WQ1
Raf	Ras binding domain (RBD)	1C1Y
PI3Kalpha	Ras binding domain (RBD)	2RD0
RalGDS	Ras-associating domain (RA)	3KH0
PLCepsilon	Ras-associating domain (RA)	2C5L
Bry2	Ras binding domain (RBD)	1K8R
RASSF5	Ras-associating domain (RA)	3DDC
AFAD RA1	Ras-associating domain 1 (RA1)	6AMB
AFAD RA1 & RA2	Ras-associating (RA) or Ras binding domain (RBD)	Model
RAIN		
RGS12		
RASSF1		
RGL1		

III. RESULTS AND DISCUSSION

3.1 Models of H - and K-Ras with their Effectors and Regulators

The crystal structures of H - and K-Ras with their regulator and effector proteins, available in PDB (Table 1), indicate that effector binding sites overlap β-sheet dimer interface of Ras [9]. Using PRISM we also identified the other regulator/effector binding regions on H - and K-Ras (Table 3). Figures 2 and 3 show the modeled complex structures of H-Ras and K-Ras4B proteins with their binding partners, respectively. After modeling the complex structures, HotRegion [54] is used to identify the hot spot residues and hot regions on modelled Ras-effector dimer interfaces (Table 4, Figures 2-4).

Table 3. H - and K-Ras complexes modeled by PRISM algorithm

Ras Isoform (PDB ID)	Binding Partner (PDB ID)	Template Interface	Binding Energy Score (BES)
H-Ras (1QRA)	AFAD RA 1 (Model)	1lfdCD	-21.71
	AFAD RA 2 (Model)	1mo1AB	-17.80
	RAIN (Model)	3iicAB	-32.06
	RGS12 (Model)	1fr3AB	-14.28
	RASSF1 (Model)	1whmAB	-32.49
	RGL1 (Model)	1fr3AB	-35.40
	CDC42 (2NGR)	2erxAB	-26.56
K-Ras (3GFT)	Sos1 (1BKD)	1nvuQS	-37.47
	p120GAP (1WQ1)	1wq1GR	-23.16
	Raf (1C1Y)	1c1yAB	-51.19
	PI3Kα (2RD0)	1lfdCD	-23.24
	RalGDS (3KH0)	1lfdCD	-45.24
	PLCε (2C5L)	1lfdCD	-37.26
	Bry2 (1K8R)	1k8rAB	-43.50
	AFAD RA1 (Model)	2pmcAB	-32.66
	AFAD RA2 (Model)	1mo1AB	-31.20
	RAIN (Model)	1lfdCD	-8.31
	RASSF5 (Nore1A) (3DDC)	1lfdCD	-51.49
	CDC42 (2NGR)	2erxAB	-18.29

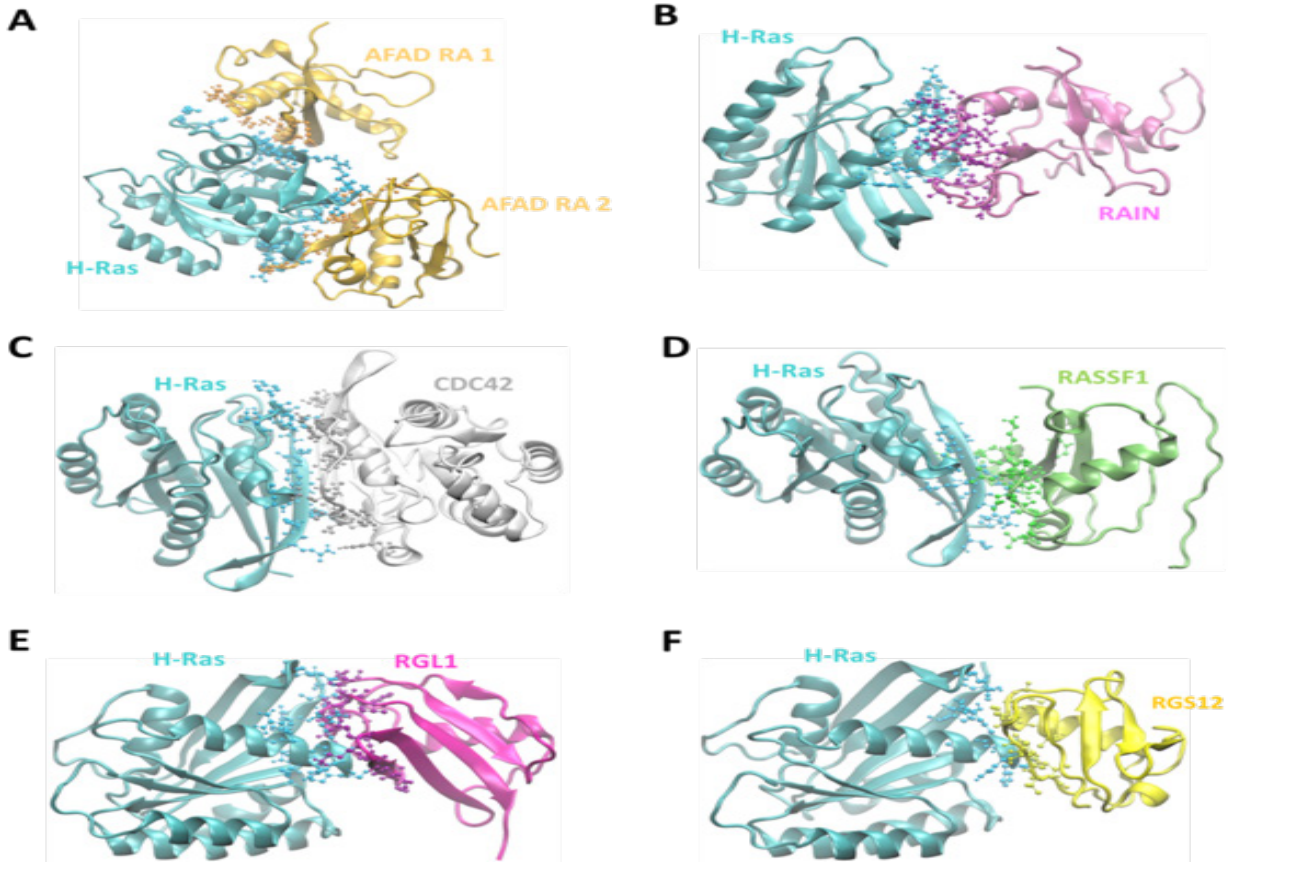

Figure 2. Modeled complex structures of H-Ras. Residues participating in the interface are shown in ball-and-stick representation. See Table 4 for interface details.

Table 4. The list of interface and hot spot residues of the modeled Ras/effector complexes. Hot spot residues are shown with an asterisk (*) and residues/domains at the allosteric lobe of Ras are underlined.

Complex (PDB IDs)	Ras residues in the interface (*hot spots)	Corresponding Ras domains in the interface	Effector residues in the interface (*hot spots)
H-Ras/AFAD RA1	E31, D33, I36*, E37, D38, S39, R41, Y64, A66, M67*	Switch I and II a3, b2	F55, T57, K58, C59*, I60*, R61, E77, K78, R80
H-Ras/AFAD RA2	M1, T2, E3, K5, T74, E76*, K104, S106, D108, P110, H166*	Switch II, Allosteric Lobe a3, a5, a7, b1, b5	S246, G247, G248, T249*, R251*, Y263*, T265, L267, D310
H-Ras/RAIN	Q22*, L23*, I24*, Q25*, N26, H27*, F28*, V29, D30, K147, T148, R149*, V152*, E153, Y157*	Switch I, Allosteric Lobe a1, a7, b6	L208*, R210, A211, H225, V238*, L241*, W242*, R243, A244, R245, P246, G247, W248, R250*
H-Ras/Cdc42	Q25*, N26, E31, D33, I36*, E37, D38, S39, Y40*, R41, K42, Q43, L52*, Y64*	Switch I and II a1, a2, b2, b3	M1, I21*, T24, T25, K27, E31, V33, V36*, F37*, D38, N39, Y40*, A41, V42, T43, T52*, Y64*
H-Ras/RASSF1	M1, E3, K5, I24*, S39, R41*, K42*, T50, D54, T74	Switch II a1, a3, b1, b2, b3	E250, A252*, R254*, H255, R261, K262, L263, L264, E267, Q268*, L272, L275*
H-Ras/RGL1	M1, T2*, E3, Y4*, E49, E76, V160*, I163*, R164*, Q165, H166	Switch II, Allosteric Lobe a7, b1	D648, E704, K706, E707, L708*, V709, F717*, Y718, A719, M720*, N721, S722*, Q723, V724
H-Ras/RGS12	T2, Y4*, E49, I163*, R164*, Q165, H166	Allosteric Lobe a7, b1, b3	S973, C974*, V975, V976*, L991*, E993, R994, H995*, G996
K-Ras/Raf	I21*, Q25, V29, D30, D33, I36, E37*, D38*, S39, Y40*, R41, D54, L56*	Switch I a1, b2, b3	T57, N64, K65, Q66*, R67, T68*, V69*, K84, V88, R89*, G90
K-Ras/PI3K p110α	I24*, Q25, D30, E31, D33, I36, E37*, D38, S39, Y40*, R41*, K42, Q43, M67, Q70	Switch I and II a1, a2, b2	W195*, N201, N202, D203, K204, Q205*, K206*, T208, K227, R230, S231, M232, L233, M282*, L792*
K-Ras/Sos1	Q22*, I24, Q25, N26*, Y32, D33, P34, T35, I36, E37, D38*, S39, Y40*, R41, L56*, H61, M67, Q70, R149*	Switch I and II, Allosteric Lobe a1, a2, b2, b3	H616, M617, Y618*, P621, N622, R625, P684, L687, R688*, N691, R694, H699, W729, S732, R739, I752, A965, E966, G969, Q973, N976, Q977
K-Ras/p120GAP	S17, I21*, Q25, H27, Y32, D33, P34, T35, I36*, E37*, D38*, S39*, Y40*, R41, H61, E63, A66, M67	Switch I and II a1, a2, b2	T785, R789*, E799, P832, S833, L902*, R903, C906*, L910*, N911, I931*, A934, K935*, Q938*, N942, V944, G947, A948, K949*, E950, Y952*
K-Ras/RalGDS	I24*, Q25, D33, P34, T35, I36*, E37, D38, S39, Y40*, R41	Switch I a1, b2	N787, L788, Y789, I801, R803, D809, N810, G811, N812, M813*, Y814*, K815, S816
K-Ras/PLCε	I24, Q25*, V29, I36, E37, D38*, S39, Y40*, R41, D54	Switch I a1, b2, b3	F2138, P2146, E2147, Q2148*, P2149, R2150, T2151, V2152*, Y2174, S2175*
K-Ras/Bry2	I36*, E37, D38, S39, Y40*, R41*, D54, M67, Q70	Switch I and II a2, b2, b3	I72, R74, G80, Q81, T82, R83*, A84, V85*, Q86, K101, D141, E156, R160, E165
K-Ras/Cdc42	Q25*, H27, E31, D33, I36, E37, D38, S39, Y40*, R41*, M67	Switch I and II a1, a2, b2	M1, T25, K27, S30, E31, V33, V36, F37, D38, N39, Y40*, A41, V42*, T43
K-Ras/RASSF5	I21*, I24, Q25*, N26, H27, V29, D30, D33, P34, T35, I36, E37*, D38, S39, Y40*, R41, S65, A66, M67, Q70	Switch I and II a1, a2, b2	E217, K218, C220, L221*, F234*, R243*, L277, P278, L279*, D280, A281*, I282, K283, Q284*, K303, F304*, M305
K-Ras/RAIN	I21*, I24*, Q25, E31, Y32, D33, P34, T35, I36, E37, D38, S39, Y40*	Switch I a1, b2	A156, S157, G158, A159*, N160, Y161*, K162*, S163, L165, E181, R182*, G184, P189
K-Ras/AFAD RA1	T87, K88, F90*, E91, H94, T124, V125*, D126, K128, Q129*, D132, L133*, S136, Y137*	Allosteric Lobe a3, a4, a5	F39, H40*, G41, V42, C59*, I60*, R61*, V62*, S63, D70, E73, T74*, E77, K78
K-Ras/AFAD RA2	M1, T2, E3*, Y4*, K5, T50, T74, E76*, K104, D108, I163*, H166, K167	Switch II, Allosteric Lobe a4, a6	S246, G247, G248, T249*, L250*, R251, Y253*, Y263, T265, L267, D310, L326*, F329*, R330, I338, L339

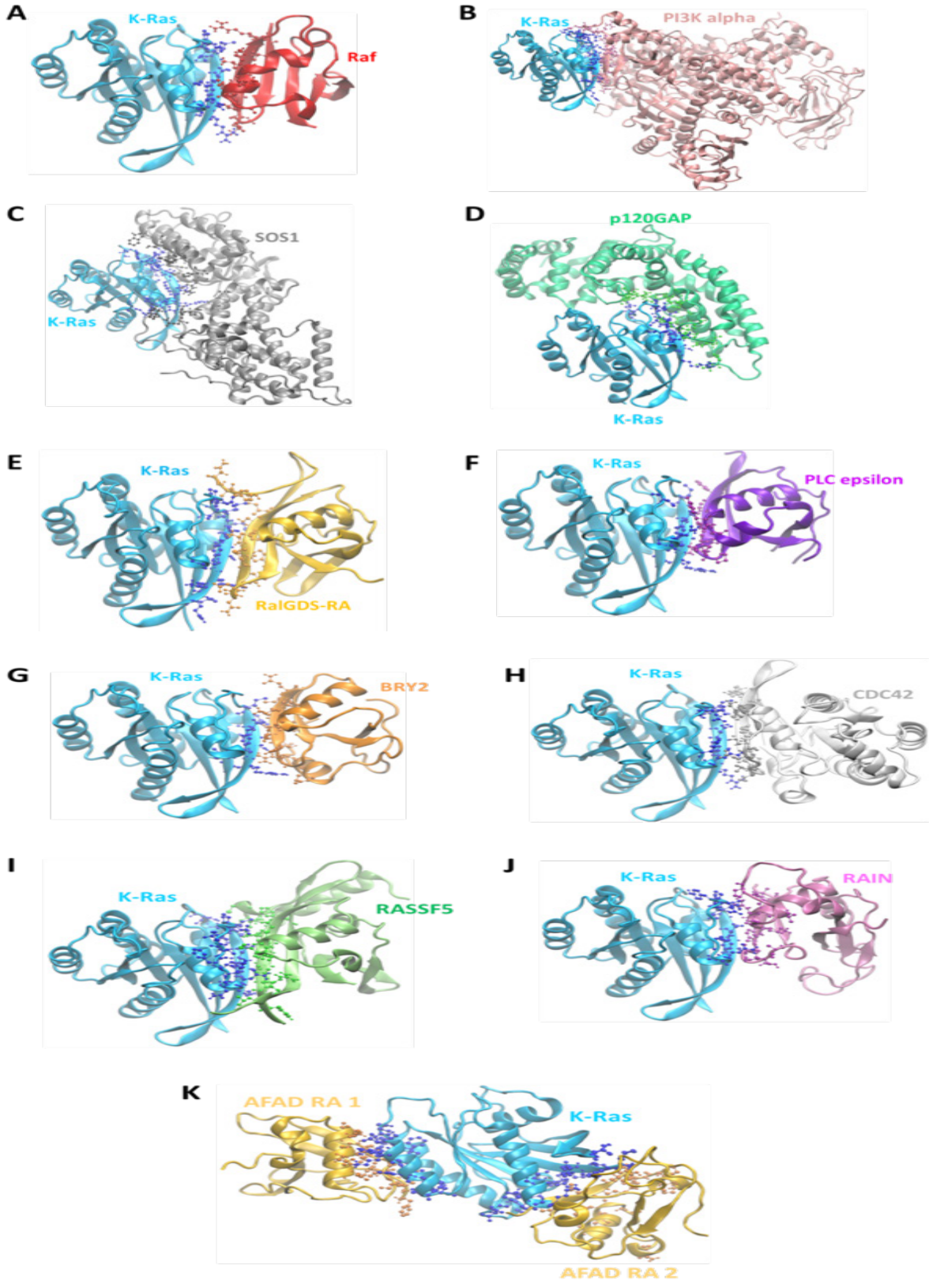

Figure 3. Modeled complex structures of K-Ras. Residues participating in the interface are shown in ball-and-stick representation. See Table 4 for interface details.

3.1.1 Case study (H-Ras/Cdc42, K-Ras4B/RAIN complexes)

One of the dimer interfaces on Ras, which is majorly populated, is β-sheet dimer interface at the effector binding site and it overlaps with the binding surfaces of effectors such as Raf, PI3K and RalGDS [9-10]. From the identified interface and hotspot residues on modeled Ras-Ras homodimer and Ras-effector heterodimer interfaces, this similarity can be deduced (Table 4) and a case study comparison is depicted in Figure 4. Ras-Ras homodimer H-Ras/Cdc42 model is aligned with the modeled complex of K-Ras4B with its effector Ras associating (RA) domain of Ras interacting protein 1 (RAIN), showing the shared binding interface and hot spots.

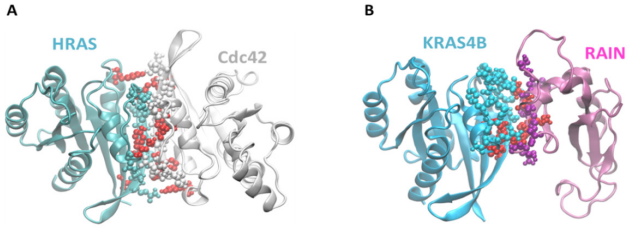

Figure 4. Comparison of Ras-Ras homodimer and Ras-effector heterodimer interfaces **A)** H-Ras (1QRA) / Cdc42 (2NGR). **B)** K-Ras4B (3GFT) / RAIN(Model). Interfaces are shown as ball-and-stick, hot spots in red.

3.1.2 Case study (Ras/RASSF, Ras/Raf complexes)

The modeling results indicate that Ras interacts with Ras association domain-containing protein (RASSF) and Raf through a similar interface, involving the β-sheet extension region (Table 4). RASSF is a potential tumor suppressor that promotes Ras induced apoptosis and cell cycle arrest [55]. Raf, on the other hand, is a proto-oncogene that induces cell proliferation. The models suggest that these two effectors with opposing functions use the same interaction surface on Ras and thus compete for same binding site to perform their function. So we may speculate that only one of the pathways is activated at a time, and the cell undergoes either an apoptosis or cell proliferation. As a case study validation of the modeling accuracy, the modeled K-Ras/C-Raf complex is compared with the experimentally determined K-Ras/A-Raf (PDB ID: 2MSE) and they are found to be perfectly aligned (with RMSD 0.557 Å) having similar interface and hot spot residues (Figure 5 and Table 5).

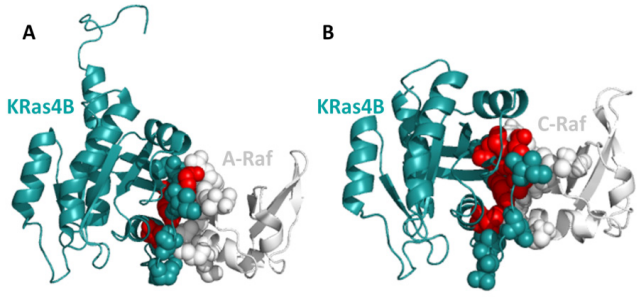

Figure 5. K-Ras/Raf complexes. **A)** K-Ras4B/A-Raf (PDB ID: 2MSE). **B)** Modeled complex: K-Ras4B (PDB ID: 3GFT)/C-Raf (PDB ID: 1C1Y) Interface residues are displayed as spheres; hot spots are shown in red.

Table 5. Interface and hot spot residue comparison of the modeled and experimentally determined KRas-Raf complexes

Complex (PDB IDs)	Ras residues in the interface (*hot spots)	Effector residues in the interface (*hot spots)
K-Ras/A-Raf (PDB ID: 2MSE)	I21*, Q25, E31, D33, I36, E37*, D38, S39, Y40*, R41, D54	T809, N816, Q818*, R819, T820, V821, K836, K839, V840*, R841
K-Ras/C-Raf (modeled)	I21*, Q25, V29, D30, D33, I36, E37*, D38*, S39, Y40*, R41, D54, L56*	T57, N64, K65, Q66*, R67, T68*, V69*, K84, V88, R89*, G90

3.1.3 Interactions of non-canonical effectors with Ras through the allosteric lobe

Most of the effectors bind Ras through effector lobe (involving mainly Switch I and partially Switch II and the β-sheet extension) and thus competes with Ras dimerization through β-sheet interface (Figure 5, Tables 4 and 5). However, importantly, some interfaces between Ras and its non-canonical effectors include residues from the allosteric lobe (the last three or four helices on Ras) and these interactions are namely, H-Ras/AFAD (Adherens Junction Formation Factor Ras associationg (RA) 2 domain); H-Ras/RAIN (Ras interacting protein also called as RasIP1); H-Ras/RGL1 (Ral guanine nucleotide dissociation stimulator-like 1); H-Ras/RGS12 (Regulator of G-protein signaling 12); K-Ras4B/AFAD RA1 and AFAD RA2.

According to the models, H-Ras interacts with RAIN with a much favorable binding energy score than K-Ras4B (Table 3, BES values – 32.06 and – 8.31). When the interface residues are analyzed, the main difference is the participation of allosteric lobe in H-Ras/RAIN but not in K-Ras4B/

RAIN complex (Table 4). Effectors such as Raf, PI3K and RalGDS are well-established effectors of plasma membrane–localized Ras but only a distinct set of Ras effectors is involved in mediating endomembrane Ras signaling and RAIN was experimentally identified as an effector recruiting to Golgi and colocalizing with Ras-GTP *in vivo* [56]. It was also shown that H-Ras and N-Ras can localize to intracellular membranes of the endoplasmic reticulum and Golgi, in addition to the extracellular plasma membrane; however, K-Ras is incapable of intracellular membrane localization [57]. Therefore, the involvement of the allosteric lobe in the interface may explain why RAIN specifically binds to H-Ras but not K-Ras. More interestingly, A146T at the allosteric lobe is the most frequent oncogenic Ras mutation after G12, G13 and Q61 [17] and it is in close proximity to H-Ras-RAIN interface which includes residues K147, T148, R149, V152, E153 and Y157 from the allosteric lobe (Table 4).

Although GTP-bound Ras interacts with AFAD/Afadin (AF6) RA1 domain, mutations at the interface that are known to block the AF6-Ras interaction did not abolish Mixed Lineage Leukemia (MLL)-AF6–mediated oncogenesis [58]. The AF6 RA1 domain rather still mediated the oncogenic self-association of MLL [58]. Thus, it is critical to understand the underlying mechanism. We modeled complexes of RA1 and RA2 domains of AF6 with H-Ras and K-Ras. The interface of the modeled H-Ras/AFAD RA1 complex agrees with the experimentally determined structure and does not involve the allosteric lobe in the interface [59]. Interestingly, however, the interfaces of the modeled H-Ras/AFAD RA2, K-Ras4B/AFAD RA1 and K-Ras4B/AFAD RA2 are largely composed of the allosteric lobe and further experimental analysis may provide more information about the functional outcome of this difference between the isoforms.

3.2 Conclusions

The recent leading approach of targeting the oncogenic Ras signaling is through the disruption of protein-protein interactions. Thus, elucidating the structural details of these protein-protein complexes is of crucial importance. However, only a small portion of Ras/effector complexes is experimentally determined and available in PDB. Computational tools for modeling protein-protein interactions help in filling the experimental gap by enriching the available structural data in literature. In this study, a powerful structural prediction algorithm, PRISM, is applied to model the interactions of H-Ras and K-Ras4B with their regulator/effector proteins. The results indicate that, expectedly, most of the effectors bind to the effector binding region that involves mainly Switch I and partially Switch II on Ras; thus competes with Ras dimerization through β-sheet interface. However, importantly, some effectors – especially the non-canonical ones – bind Ras through the allosteric region involving the last three or four helices on Ras and overlap with the helical Ras dimerization interface, suggesting a role for the "allosteric lobe" that could guide therapeutic studies targeting oncogenic Ras signaling.

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