American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Mini Review

K-Ras Plasma Membrane Interactions: A Tractable Therapeutic Target

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To Cite This Article: *Kwang-jin Cho. K-Ras Plasma Membrane Interactions: A Tractable Therapeutic Target. 2020 - 9(5). AJBSR.MS.ID.001440.*

DOI: 10.34297/AJBSR.2020.09.001440.

Received: June 30, 2020; **Published:** July 31, 2020

Abstract

Ras proteins are small GTPases that function like a molecular switch regulating cell proliferation, survival and differentiation at the plasma membrane (PM). Of the three major Ras isoforms, constitutively active mutations in K-Ras are frequently found in human cancers. Despite its critical role in tumorigenesis, no anti-K-Ras therapies are currently available in clinic. One strategy for blocking oncogenic K-Ras activity is to disrupt K-Ras interaction with the PM since K-Ras must primarily interact with the PM for its biological activity. This review will provide insights into recently reported molecular mechanisms that regulate K-Ras from the PM, which can be targeted to disrupt oncogenic K-Ras signaling.

Ras small GTPases are central regulators of cell proliferation, differentiation, survival and apoptosis. Consistent with this key regulatory role, constitutively active mutations of Ras are present in ~19% of human cancers [1]. There are three ubiquitously expressed Ras isoforms in mammalian cells: H-, N- and K-Ras (two splice variants, K-Ras4A and K-Ras4B), and mutations of the K-Ras isoform are found in ~88% of pancreatic, ~50% of colorectal, and ~32% of all lung cancers [1]. Thus, anti-K-Ras therapies would have great clinical utility. There are four approaches currently being pursued for developing therapies for K-Ras-driven cancers: 1) dissociation of K-Ras from the plasma membrane (PM), 2) direct allosteric inhibition of K-Ras, 3) inhibition of K-Ras downstream effectors, and 4) dysregulation of cell metabolism [2,3]. This review will focus on recent strategies for dissociating K-Ras from the PM, which results in inhibition of K-Ras signaling.

Phosphatidylserine Depletion from the inner Pm Leaflet Dissociates K-Ras from the PM and Blocks Oncogenic K-Ras Signaling

Many studies clearly demonstrate that Ras proteins must bind primarily to the inner leaflet of the PM for its biological activity and blocking Ras/PM interaction results in inhibition of Ras signaling [4,5]. For a stable PM interaction, newly synthesized Ras proteins must undergo a series of post-translational modifications at the C-terminal CAAX motif (where C = Cys, A = aliphatic amino acids, and X = Met or Ser). First, a cytosolic farnesyltransferase attaches a farnesyl group to the Cys, which allows Ras to bind to the cytosolic leaflet of the ER [6,7]. RCE1 (Ras converting CAAX endopeptidase 1) then removes the AAX tripeptide, followed by the methylation of the now C-terminal prenylated Cys by ICMT (isoprenylcysteine carboxyl methyltransferase) [8,6]. N-, H-, and K-Ras4A are further modified with the addition of palmitic acids on one or two other Cys residues near the prenylated Cys [7], allowing the stable localization to the PM. K-Ras4B (hereafter, K-Ras) has a single farnesyl lipid moiety preceded by six Lys residues, called a polybasic domain [9], and the strong positive charge of this polybasic domain allows K-Ras to interact with anionic phospholipids in the PM through electrostatic interaction [10,11].

Phosphatidylserine (PtdSer) is an anionic phospholipid enriched in the inner PM leaflet. Its charged head group confers a strong negative electrostatic potential to the cytosolic face of the

PM. K-Ras interacts with PM PtdSer through the combined effects of the C-terminal polybasic domain and the farnesyl chain of the lipid anchor, which together provide high selectivity for PtdSer over other anionic phospholipids. Depletion of PM PtdSer dissociates K-Ras from the PM and blocks oncogenic K-Ras signaling in vivo and in vitro [12, 13, 14, 15, and 16], suggesting its essential role in K-Ras biological activity. Several molecular mechanisms that maintain PtdSer enrichment at the inner PM leaflet have been reported.

Perturbation of sphingomyelin metabolism depletes PM PtdSer content

Recent studies have demonstrated that acid and neutral sphingomyelinases (SMases) that convert sphingomyelin to ceramide in the lysosome and ER, respectively, regulate the PM localization of PtdSer and K-Ras. Acid SMase inhibitors including tricyclic anti-depressants mislocalize PtdSer and K-Ras from the PM to endo membranes and block oncogenic K-Ras signaling [13,16,17]. Replenishing PM PtdSer with exogenous PtdSer supplementation in these cells restores K-Ras PM binding and signaling, suggesting that the K-Ras PM mislocalization is through PtdSer depletion at the inner PM leaflet [13,16,17]. Furthermore, supplementation with recombinant Acid SMase returns PtdSer and K-Ras to the PM in Acid SMase-inhibited cells, suggesting that Acid SMase regulates PtdSer localization at the PM, resulting in K-Ras PM interaction [16]. In addition, inhibition of neutral SMase mislocalizes PtdSer and K-Ras from the PM and blocks oncogenic K-Ras signaling, and supplementation with exogenous PtdSer in these cells restores K-Ras PM localization [18]. Taken together, these studies suggest that acid and neutral SMases regulate oncogenic K-Ras signaling through maintaining PtdSer enrichment at the inner PM leaflet, and that acid and neutral SMases are tractable targets for the development of anti-K-Ras therapies.

Phosphatidylinositol 4-phosphate regulates PtdSer transport from the ER to the PM

Phosphatidylinositol 4-phosphate (PI4P) is synthesized from PI at the PM and Golgi complex by four PI 4-kinases in mammalian cells: PI4K IIα and β (PI4K2A and 2B), and PI4K IIIα and β (PI4KA and PI4KB) [19]. PI4KA and 2B are predominantly localized to the PM, while PI4K2A and PI4KB localize to the Golgi complex [19]. Recent studies have reported that PI4P content at the PM and Golgi complex regulate the PM localization of PtdSer and K-Ras. In mammalian cells, oxysterol-binding protein-related protein (ORP) 5 and 8 are recruited to ER-PM membrane contacting sites (MCSs) to exchange newly synthesized PtdSer from the ER for PI4P from the PM [20, 21].

This exchange is driven by PM PI4P synthesis by PI4KA and the concomitant hydrolysis of PI4P by Sac1 phosphatase in the ER to maintain a PI4P gradient across the PM and ER. Eliminating any component of this machinery depletes PtdSer content at the inner PM leaflet and mislocalizes K-Ras from the PM [22, 23]. PI4KA inhibition further blocks growth of multiple K-Ras-driven cancer cells in vitro [23]. Moreover, Golgi PI4P depletion by glucose starvation or PI4KB knockout redistributes PtdSer from the PM to endomembranes, resulting in K-Ras translocation to mitochondria and inhibited oncogenic K-Ras signaling [24]. These studies suggest that molecular mechanisms that maintain PI4P content at the PM and Golgi complex is a plausible target for blocking oncogenic K-Ras signaling.

K-Ras phosphorylation Dissociates K-Ras from the PM and Abrogates Oncogenic K-Ras Signaling

Protein kinase C (PKC) phosphorylates K-Ras blocking oncogenic K-Ras signaling

Another mechanism to dissociate K-Ras from the PM and block oncogenic K-Ras signaling is K-Ras phosphorylation by PKC. PKC directly phosphorylates K-Ras at Ser181 and to a lesser extent at Ser171 and Thr183, redistributing K-Ras from the PM to the endomembranes including mitochondria, where it triggers enhanced apoptosis [25]. PKC activators can further suppress the growth of K-Ras tumors in nude mice by stimulating K-Ras phosphorylation [25,26]. A recent study further demonstrated that chalcone-based small molecules stimulate PKC, which phosphorylates K-Ras at Ser181, resulting in K-Ras PM dissociation and inhibition of K-Ras-driven human cancer cell growth [27]. There are three classes of PKC isozymes: conventional (α, β, γ), novel (δ, ε, η, θ), and atypical (ζ, ι). Several studies propose that PKCδ is a suppressor for oncogenic K-Ras. PKCδ protein levels are lower in endometrial cancer cells harboring oncogenic mutant K-Ras than WT K-Ras [28], and approximately 40% of PKCδ loss-of-function mutations are found in pancreatic cancers harboring oncogenic mutant K-Ras [29]. Moreover, patients with non-small cell lung cancers expressing oncogenic mutant K-Ras show an increased overall survival rate when they also have higher PKCδ mRNA levels [30]. Taken together, these studies suggest that PKCδ may have an anti-cancer activity in cancers expressing oncogenic mutant K-Ras possibly by dissociating oncogenic mutant K-Ras from the PM and thereby, abrogating oncogenic K-Ras signaling.

Stimulating the AMPK/eNOS/PKG2 pathway phosphorylates K-Ras, blocking oncogenic K-Ras signaling

In addition to PKC, cGMP-dependent protein kinase 2 (PKG2) has been identified as a new K-Ras kinase phosphorylating K-Ras at Ser181 through the AMPK/eNOS/PKG2 signaling pathway [13]. A recent study demonstrated that direct or indirect activation of AMP-activated protein kinase (AMPK) stimulates the activity of endothelial nitric oxide synthase (eNOS), one of the AMPK

downstream effectors. This in turn, elevates cellular nitric oxide levels, which promotes soluble guanylyl cyclase (sGC) activity, generating cGMP from GTP. Upon cGMP binding, activated PKG2 [31,32], but not PKG1, is recruited to the PM and phosphorylates K-Ras at Ser181, resulting in K-Ras dissociation from the PM. Moreover, stimulation of the AMPK/eNOS/PKG pathway shows anti-cancer activity. Chronic activation of components in the signaling pathway by pharmacological agents inhibits the growth of non-small cell lung cancer cells expressing oncogenic mutant K-Ras [13].

Conclusion

Oncogenic mutant K-Ras is found in approximately 14% of all human cancers, but no anti-K-Ras therapies are available in clinic. Despite the critical role of K-Ras interaction with the PM for its biological activity, the exact molecular mechanisms of K-Ras transport to and maintenance at the PM are not fully understood. Investigating these mechanisms will provide insights into novel approaches of developing anti-K-Ras therapeutics.

Acknowledgement

This work was supported by the National Cancer Institute [R00-CA188593 to K.-J.C.].

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