Review

Molecular Cancer Research

The Key Role of Calmodulin in *KRAS*-Driven Adenocarcinomas

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Abstract

KRAS4B is a highly oncogenic splice variant of the KRAS isoform. It is the only isoform associated with initiation of adenocarcinomas. Insight into why and how KRAS4B can mediate ductal adenocarcinomas, particularly of the pancreas, is vastly important for its therapeutics. Here we point out the overlooked critical role of calmodulin (CaM). Calmodulin selectively binds to GTP-bound K-Ras4B; but not to other Ras isoforms. Cell proliferation and growth require the MAPK (Raf/MEK/ERK) and PI3K/Akt pathways. We propose that Ca^{2+}/calmodulin promote PI3Kα/Akt signaling, and suggest how. The elevated calcium levels clinically observed in adenocarcinomas may explain calmodulin's involvement in recruiting and stimulating PI3Kα through interaction with its n/cSH2 domains as well as K-Ras4B; importantly, it also explains why K-Ras4B specifically is a key player in ductal carcinomas, such as pancreatic (PDAC), colorectal (CRC), and lung cancers. We hypothesize that calmodulin recruits and helps activate PI3Kα at the membrane, and that this is the likely reason for Ca^{2+}/calmodulin dependence in adenocarcinomas. Calmodulin can contribute to initiation/progression of ductal cancers via both PI3Kα/Akt and Raf/MEK/ERK pathways. Blocking the K-Ras4B/MAPK pathway and calmodulin/PI3Kα binding in a K-Ras4B/calmodulin/PI3Kα trimer could be a promising adenocarcinoma-specific therapeutic strategy. *Mol Cancer Res; 13(9); 1265–73. ©2015 AACR.*

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Introduction

RAS signaling cascades are still not entirely understood (1). Cell decisions are temporal, and functions typically involve more than one pathway. Growth and proliferation, which require both the mitogen-activated Ras/Raf/MEK/ERK (MAPK) and the phosphoinositide-3-kinase (PI3K)/Akt pathways, provide a compelling example (2, 3). Under normal physiologic conditions, PI3Kα is recruited to the membrane by activated tyrosine kinase receptors (RTK) such as the EGFR or adaptor proteins. When K-Ras4B is constitutively activated by mutations, calmodulin can act to accomplish the full activation of the PI3K/Akt pathway role. K-Ras4B is the only Ras isoform or splice variant to bind calmodulin; we propose that by activating the PI3Kα/Akt pathway in the absence of a growth cue, calmodulin plays a critical role in adenocarcinomas, particularly pancreatic cancer. The high calcium levels observed in adenocarcinomas may explain calmodulin's involvement in recruiting and activating PI3Kα through interaction with its n/cSH2 domains as well as K-Ras, and why K-Ras4B specifically is a key player in these cancers. Calmodulin's role in recruiting PI3Kα essentially makes it act as a Ca^{2+}-regulated scaffolding protein (4). On the basis of genetically engineered mouse models, even in the absence of RTK signal, oncogenic mutations in *KRAS* can lead to oncogene-induced senescence or to proliferation and differentiation (5); however, on their own, oncogenic mutations in K-Ras4B are unable to achieve full PI3Kα activation. Thus, a compelling question is whether in addition to the mutations, there exists another factor. Possible factors include elevated levels of calmodulin/Ca^{2+}, a redundant pathway, bypassing PI3Kα-dependent growth, and PI3Kα mutations. The first two can be cell/tissue-specific. A K-Ras4B/calmodulin/PI3Kα trimer fits available experimental and clinical data, is able to explain the high frequency of oncogenic K-Ras4B in adenocarcinomas, particularly in pancreatic cancer, and is a promising, highly specific therapeutic venue for adenocarcinoma.

Ras Isoforms, Mutations, and Cancer

Ras proteins regulate cell proliferation, differentiation, survival, migration, and apoptosis. H-Ras, N-Ras, K-Ras4A, and K-Ras4B (6, 7) are highly homologous in sequence (~80%). They are distinguished mostly by their C-terminal hypervariable regions (HVR). They are preferentially located at different membrane microdomains (8) and are not functionally redundant (9–20). *KRAS* oncogene has been implicated in malignancies of the lung, pancreas, and colon. Activating *KRAS* mutations, are present in approximately 90% of the cases of human pancreatic ductal adenocarcinoma (PDAC), the predominant form of pancreatic cancer (21–28). The *KRAS* oncogene is mutated in approximately 50% of colorectal cancers (29–31). Oncogenic *KRAS* has also been implicated in non–small cell lung carcinoma (NSCLC; 32). PDAC is complex and heterogeneous (26, 33–38) and the key mutations may differ

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(22, 26, 39–43). It is largely driven by the K-Ras4B splice variant of the *KRAS* gene (43).

Distinct Signaling Pathways in *KRAS*-Driven Adenocarcinomas

Oncogenic K-Ras signaling in PDAC is complex and dynamic (44–46). It involves three major pathways: Raf/MEK/ERK, PI3K/Pdk1/Akt, and the Ral guanine nucleotide exchange factor (RalGEF; refs. 43, 47–50). PDAC initiation, progression, and maintenance depend on K-Ras/PI3K/Pdk1/Akt signaling. This is supported by treatment of primary acinar cells from human pancreas with PI3K/Pdk1/Akt pathway inhibitors (50). Like $KRAS^{G12D}$-driven PDAC, pancreas-specific expression of $PIK3CA^{H1047R}$ ($p110\alpha^{H1047R}$, a constitutively active oncogenic class IA PI3K), selectively activates the PI3K/Pdk1/Akt pathway, indicating that the constitutively activated pathway can induce acinar-to-ductal metaplasia, pancreatic intraepithelial lesions (PanIN), and PDAC (43, 50); inactivation of Pdk1 blocked tumor development and progression, confirming the key involvement of PI3K pathway activation in *KRAS*–driven PDAC, although these findings are in contrast to Raf/MEK/ERK being considered as the dominant signaling pathway (49). Activation of the MAPK pathway can drive pancreatic neoplastic changes, indicating that both pathways operate in adenocarcinoma development. Mutant RalGEFs are important Ras effectors particularly in *KRAS*-driven PDAC and colon cancers (37, 51–53), and are consistently highly activated in pancreatic tumors (54, 55). RalA acts in early and RalB in late PDAC stages. The two mutant isoforms reflect compensatory short-term versus prolonged loss of Ral function (51, 56). In colorectal tumor cells, loss of one Ral isoform increases GTP loading of the other.

K-Ras exploits different downstream effectors or isoforms in PDAC and NSCLC (50). The contribution of B-Raf to *KRAS*–driven pancreatic carcinogenesis is unclear; C-Raf is required in $KRAS^{G12D}$-driven NSCLC, but apparently has no role in $KRAS^{G12D}$-driven pancreatic carcinogenesis (43, 50, 57, 58). $KRAS^{G12D}$–driven PDAC requires PI3K/Pdk1/Akt signaling; $KRAS^{G12D}$-driven NSCLC is unaffected by loss of Pdk1 (50), suggesting differential activation of the PI3K/p110 isoform in NSCLC. In support of this, earlier pharmacologic studies on PDAC and NSCLC observed tissue-specific differences in K-Ras signaling (59). Although abolishing the K-Ras/PIK3CA interaction significantly diminished *KRAS*–driven NSCLC *in vivo*, class IA PI3K inhibitor or p110α isoform-specific inhibitor alone were only mildly effective (60); when combined with a MEK1/2 inhibitor, the tumor size decreased significantly (59, 60). In contrast, in pancreatic cancer, a class IA PI3K inhibitor reduced considerably tumor progression in $KRAS^{G12D}$–driven PDAC *in vivo* (50). RTK signal activation in K-Ras–mutant tumors also differed: unlike the inhibitory effect of $KRAS^{G12D}$–driven PDAC initiation by EGFR deletion, no effect was observed in $KRAS^{G12D}$–driven NSCLC (61). Thus, similar to *BRAF*–driven melanoma and colon cancer which differ in their response to targeted therapies (62, 63), K-Ras signaling in NSCLC and PDAC also differ, indicating the need for adenocarcinoma-specific treatment. Below we suggest that the difference lies in the involvement of K-Ras4B splice variant, whose farnesylated HVR is uniquely regulated by Ca^{2+}-bound calmodulin in ductal adenocarcinomas. Calmodulin could be the missing key to understand K-Ras4B MAPK and PI3K/Pdk1/Akt pathway regulation.

Calmodulin/Ca^{2+} Modulate Specifically the Activation of MAPK and PI3K/Akt Pathways by K-Ras4B in Ductal Adenocarcinomas

Calmodulin, a small Ca^{2+}-binding protein (64), acts in signal transduction in cell growth, differentiation, proliferation, survival, and motility (65, 66) through association with calmodulin-binding proteins (65, 67). Calmodulin plays key roles in processes in cancer biology and associated signaling pathways (68). Recent evidence suggests that Ca^{2+}/calmodulin selectively modulate K-Ras4B signaling. Unlike other isoforms, GTP-loaded K-Ras4B can interact with calmodulin in a Ca^{2+}-dependent manner (69–72). In fibroblasts, calmodulin binding temporarily downregulates the Ras/Raf/MEK/ERK (69) and upregulates the Ras/PI3K/Akt pathway (ref. 73; Fig. 1). Farnesylated HVR is required for calmodulin's isoform-selective actions (70–72, 74). Though the HVR is calmodulin's primary binding site with the farnesyl docked into calmodulin's hydrophobic pocket (72), HVR's inability to fully mimic the calmodulin/K-$Ras4B_{1-188}$ interactions suggests catalytic domain involvement (72). Calmodulin downregulates the ERK1/2 pathway at low serum concentration (69, 75); calmodulin's inhibition preferentially activates the Raf/MEK/ERK pathway.

Raf/MEK/ERK and PI3K/Akt pathways are often deregulated in cancer. Mutations in genes that encode components of these pathways occur at high frequency (76). Mutations in Ras genes affect both. *KRAS* is altered in approximately 90% of human PDAC (21) cases, 50% of colorectal cancers (29, 30), and 30% of lung cancers (32). The fact that constitutive activation of the K-Ras causes adenocarcinomas through these two major pathways points to Ca^{2+}/calmodulin involvement. Calmodulin enhances cell proliferation. Highly sustained activation of the ERK pathway induces overexpression of $p21^{cip1}$, a cyclin-dependent kinase inhibitor 1, which in turn leads to growth arrest of the cells, while transient activation followed by a sustained but lower level of ERK activity induces cell proliferation in many systems (77–80). Calmodulin prevents a too-sustained ERK1/2 activation and cell proliferation upon growth factor stimulation (69, 75) and promotes growth through the Ras/PI3K/Akt pathway.

The data above together with the observation that calmodulin is upregulated in many cancers including colorectal (81) and lung (82) adenocarcinomas support calmodulin's key role in cancer initiation and progression. Considerable data also support the involvement of calcium in adenocarcinomas. S100 calcium–binding protein P (S100P), a Ca^{2+}-binding protein associated with the progression of several cancer types including pancreatic, prostate, NSCLC, breast, and colorectal (83) has been implicated in migration, invasion, proliferation, and survival of cancer cells *in vitro* and increased tumor growth *in vivo*. Upregulation of S100P is an early event in pancreatic cancer development and its expression increases throughout the progression of PanIN to invasive PDAC. S100P was observed in 95% of the cases of PDACs (84). S100P-containing staining patterns are suggestive of PDAC, and S100P was proposed a promising diagnostic marker in pancreatic cancer screening (85, 86). S100P binds and regulates IQGAP1 (87) as does calmodulin (88). Akt is also IQGAP1's partner as are many others. IQGAP1 is known to induce EGF-stimulated Akt-mediated proliferation (89). Regulation of Ca^{2+} responses

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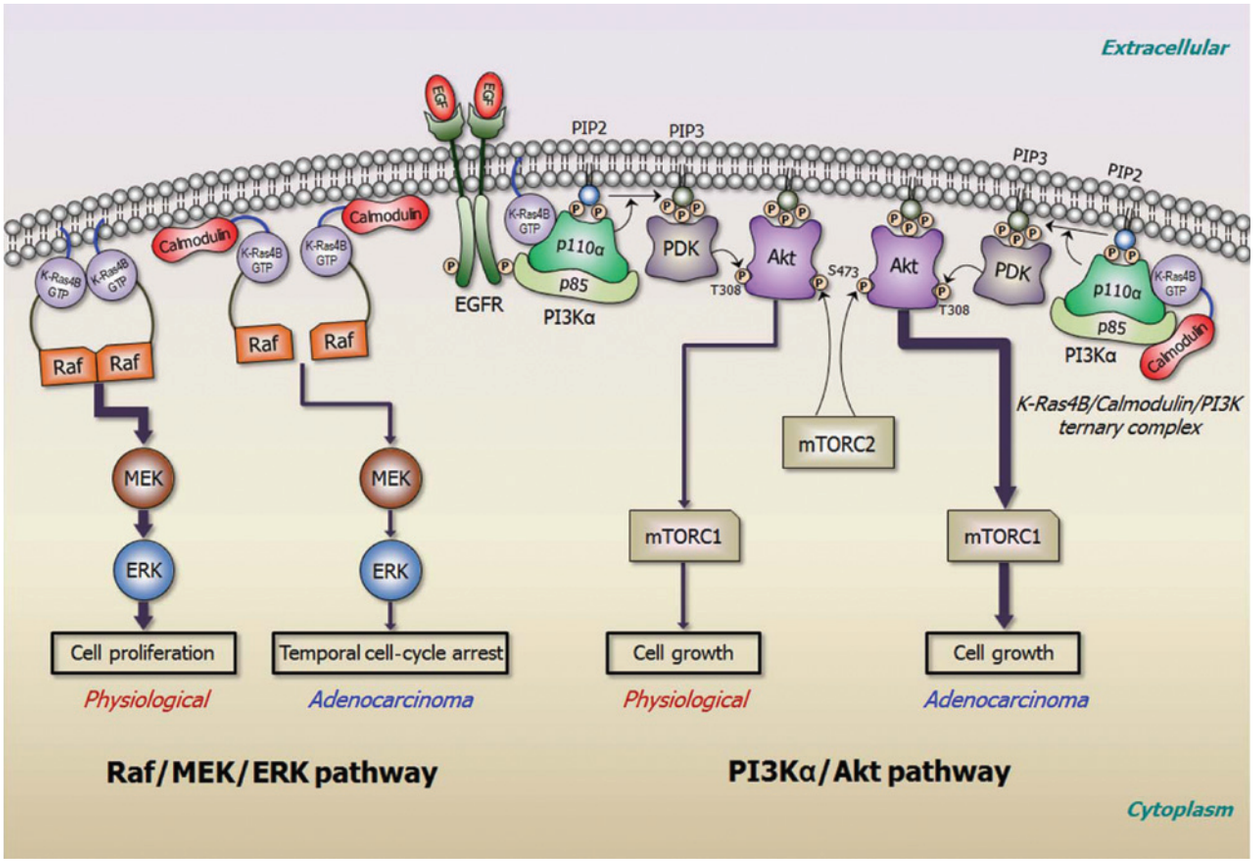

Figure 1.
Differential effects of calmodulin on two key K-Ras4B effector pathways. Calmodulin (CaM) binding to K-Ras4B-GTP downregulates the Raf/MAPK kinase/extracellular signal-regulated kinase (Raf/MEK/ERK) pathway. Raf/MAPK signaling and calmodulin's upregulation of PI3K result in proliferative effect through inducing the expression of cyclin D1, a G_1–S–specific cyclin, which is essential for cell-cycle progression. Cyclin D1/cyclin-dependent kinase 4 (CDK4) complex phosphorylates and inhibits retinoblastoma (RB) protein and regulates G_1–S transition. Binding of calmodulin to K-Ras4B-GTP activates the PI3K/Akt (also known as protein kinase B, PKB) pathway and enhances cell migration through inducing the expression of matrix metalloproteinase 2 (MMP-2) which breaks down type IV collagen, a major structural component of basement membranes. Thus, MMPs play crucial roles in invasion and metastasis.

influences migration of pancreatic cancer cells (90). The relationship between Ca^{2+} and adenocarcinomas has even led to the suspicion that excess cytosolic calcium can be associated with the disease, a hypothesis that recently has been proven groundless (91). Blocking some calcium channels resulted in antiproliferative action of adenocarcinomas (92) and inhibition or knockdown of calcium release–activated calcium modulator Orai3 channel reduced store operated calcium entry and inhibited cell proliferation lung adenocarcinoma (93). Elevated Ca^{2+} levels in ovarian adenocarcinoma cells reduced proliferation as compared with other tumor types.

A growing body of evidence in the literature indicates that calmodulin is upregulated in ductal cancers. As we noted above, calmodulin binding to K-Ras4B promotes the two characteristics of cancer: cell proliferation and migration via the MAPK and Akt pathways. Taken together, these results suggest a role for calmodulin in the initiation and progression of pancreatic, colorectal and lung cancers in agreement with the body of clinical data of high Ca^{2+} in K-Ras4B–dependent cancers (94). Calmodulin temporarily forestalls Raf and MAPK and promotes PI3K/Akt activation, proliferative signaling, and cell migration.

Below, we outline a possible mechanism for the modulation of the Raf/MAPK and PI3K/Akt pathways by calmodulin/K-Ras4B. Liao and colleagues (73) suggested that ternary complex formation between K-Ras4B, calmodulin and PI3K p110 might lead to an increase in the activity of Akt. This can be the case for the p110γ isoform. PI3Kα is more likely to bind calmodulin through p85 SH2 domains (Fig. 1), absent in PI3Kγ as indeed observed by Joyal and colleagues (95).

A Structural View Supports Calmodulin's Involvement in Adenocarcinomas

Direct physical interaction between Raf kinase and signal-activated Ras promotes Raf side-to-side dimerization (96) and Raf/MEK/ERK pathway activation (97). Ras is believed to function as a monomer; however, as signaling requires Raf's dimerization, it has been suspected that Ras also dimerizes. Binding of active Ras dimers (98) to Raf monomers recruits the Raf/14-3-3 complex to the plasma membrane and induces conformational changes that initiate molecular rearrangements and multiple phosphorylation events, which in turn enhance Ras/Raf binding (99) and stabilize Ras-mediated Raf activation (100). Ca^{2+}-dependent calmodulin/

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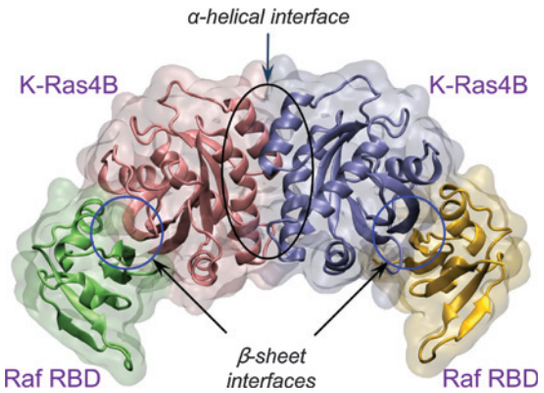

Figure 2.
Predicted tetrameric complex of K-Ras4B-GTP/Raf1-RBD. K-Ras4B-GTP homodimer interfaces (ref. 98; ice blue and pink) binds RBD of Raf1 monomers (green and yellow). Raf1 RBD is known to bind to Ras through the β-sheet (at the switch I and effector binding region) interface (PDB ID: 4G0N). Thus, K-Ras4B can dimerize through the helical interface (α3 and α4) so that each K-Ras4B monomer can bind to Raf1 RBD and promote Raf dimerization and activation. To construct a model for the tetramer complex of Raf/K-Ras, we used PRISM (105–107), an efficient template-based algorithm. PRISM predicts the structures of protein complexes by utilizing the structural similarity between template experimental interfaces and target surfaces. Here, we obtained the structural data from the PDB. K-Ras catalytic domain has 10 structures in the PDB. They include GDP- and GTP-bound conformations. We used the GTP-loaded active K-Ras molecules in the Ras dimer predictions.

K-Ras4B binding can promote K-Ras4B dissociation from the membrane (101), affect Raf's recruitment, and interfere with K-Ras dimerization. Our structural analysis suggests two possible major interface classes for K-Ras4B homodimerization: β-sheet (at the switch I and effector binding region) and helical (primarily α3 and α4) interfaces. Raf's dimerization is likely if K-Ras4B dimerizes through the helical interface (Fig. 2). High-affinity calmodulin binding to the farnesylated HVR may prevent recruitment of Raf to the plasma membrane and downregulate Raf's activation (Fig. 1). Presumably, only a small fraction of the active K-Ras4B binds calmodulin, allowing a low level of Raf activity.

Experiments with K-Ras4B–negative fibroblasts indicate that Akt growth factor–dependent cell migration and activation requires K-Ras4B. The inability of K-Ras4A or oncogenic N-Ras to restore K-Ras4B function in these cells suggests the involvement of a unique binding partner (73). The only known protein that fits this description is calmodulin (95, 102). Cells treated with calmodulin antagonists phenocopied the biologic outcomes of K-Ras4B–negative cells, failed to activate Akt and induce migratory response through matrix metalloproteinase 2 (MMP-2) expression. MMP-2 is involved in the breakdown of type IV collagen and induces cell detachment, migration, and metastasis of invasive tumors. MMP-2 levels are elevated in breast, brain, ovarian, pancreas, colorectal, bladder, prostate and lung cancers, and melanoma (103). Treating cells with PI3K or Akt inhibitors confirmed that the transcriptional activity of the *MMP-2* gene is specifically controlled by K-Ras4B through a PI3K/Akt-dependent signaling pathway (104). Taken together, these results indicate that the K-Ras4B/calmodulin complex along with Ca^{2+} is the driving force behind growth factor–dependent Akt activation and that the PI3K/Akt pathway is essential for migratory activity. The fact that the K-Ras4B/calmodulin complex and PI3K are involved in Akt activation and the observation that calmodulin can directly activate PI3K (95), support the notion of a ternary complex between K-Ras4B, calmodulin, and PI3Kα suggested by Liao and colleagues (73), albeit not necessarily with p110. Exploiting the powerful template-based protein–protein complex structure prediction algorithm PRISM (105–107), we modeled the binary interactions of PI3K p110α catalytic subunit with GTP-loaded K-Ras4B, and the Ca^{2+}/calmodulin interaction with the PI3K p85α cSH2 domain, in agreement with the earlier indications from Joyal and colleagues experiments (95). A possible model of a ternary complex between K-Ras4B, calmodulin, and PI3K p110/p85 is shown in Fig. 3. calmodulin's binding to the cSH2 and nSH2 domains of p85 are more stable than to p110 and is in line with PI3K activation scenario (108), as detailed below. While the model may not reflect accurately the interaction details, it nevertheless not only supports the idea of ternary complex formation, but also indicates that calmodulin may indeed have a key function in Akt activation.

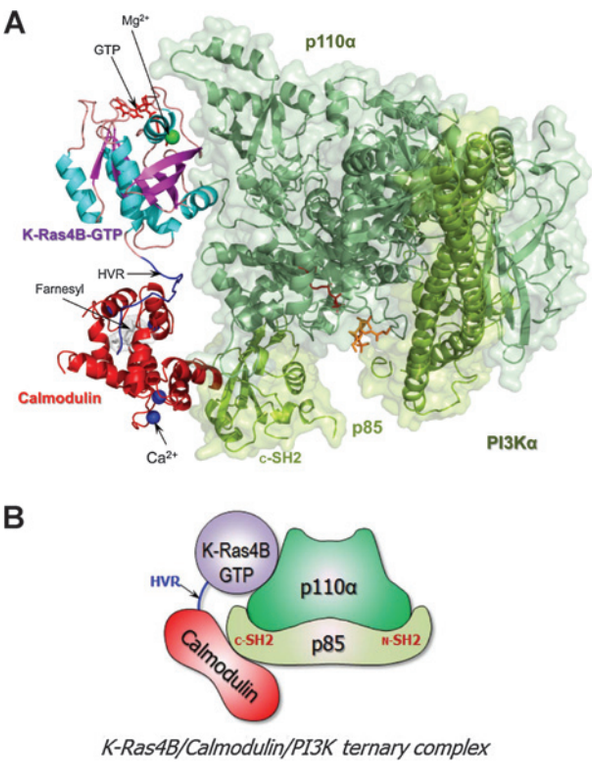

Figure 3.
A K-Ras4B-GTP/calmodulin/PI3Kα ternary complex model based on the prediction. We used the G-domain of K-Ras (166 residues), full-length calmodulin (149 residues), and the p110 catalytic p85 regulatory subunits of PI3K as target proteins. Full-length calmodulin has about 75 structures in the PDB. We considered only X-ray structures with <3.00 Å resolution. In this way, we reduced the number of calmodulin structures to 43 with 71 chains in total. PI3K p110 catalytic domain has 4 isoforms, p110α, p110β, p110γ, and p110δ. We used the p110α and p110γ structures in the PDB. We predicted models for the binary interactions between K-Ras and PI3K, and calmodulin and PI3K. We identified the contact regions using HotRegion (131). HotRegion is a database of predicted hot spot clusters. It identifies the regions that are important for the stability of protein complexes by using predicted hot spot residues, major contributors to the binding energy. Then, we built a model for the ternary complex based on the binary interactions and available literature data. A, the detailed structure; B, a simplified cartoon rendering for clarity.

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Activated Ras can directly bind p110 and activate PI3K; however, the dissociation constant, K_d, for the Ras–PI3K complex is higher than the 160 nmol/L K_d for the Ras–Raf RBD complex (109, 110) and the 1 μmol/L K_d for the Ras-Ral guanine nucleotide dissociation stimulator (RalGDS) RBD complex (111) indicating that the Ras-binding domain of PI3K p110 has a relatively lower affinity for Ras. This suggests a significant role for calmodulin in ternary complex formation and PI3K activation. Calmodulin binding might allosterically induce a conformational change in the RBD of PI3K in a way that cooperatively increases the affinity of K-Ras for PI3K, extending the duration of PI3K/Akt signaling. The low binding affinity of p110/K-Ras4B and the catalytic enhancement (8- to 10-fold) of p110 by GTP-bound K-Ras (19) highlight the importance of membrane localization of p110α via p85 nSH2 domain binding to the phosphorylated tyrosine of RTKs (or GPCR or cytokine receptors for p110γ) or their associated adaptor proteins.

Coimmunoprecipitation and affinity chromatography suggested that calmodulin/Ca^{2+} binds p85; this was further affirmed by CGS9343B, a calmodulin antagonist that inhibited basal and Ca^{2+}-stimulated phosphorylation of phosphatidylinositol in intact cells (95). While no direct affinity measurements are available, we expect that calmodulin/Ca^{2+} bind to the cSH2-p85 with much higher affinity than to the nSH2-p85. The phosphorylated insulin receptor substrate-1 (IRS-1) peptide KKHTDDGYMPMSPGVA (residues 605–615) with the ^{P}YXXM motif can disrupt the cSH2/calmodulin/Ca^{2+} binding. Calmodulin stimulates PI3Kα phosphorylation of phosphatidylinositol (PtdIns) to PtdIns-3-P or PtdIns-4-P; but not PtdIns-4,5-P2 to PtdIns-3,4,5-P3. In the experiment conducted by Joyal and colleagues, a concentration of 2 and 5 μmol/L calmodulin with 100 μmol/L Ca^{2+} in a Chinese hamster ovary cell line showed only 10% and 50% stimulation of PI3K activity, respectively (95). These data imply that calmodulin might only have a high micromolar affinity to cSH2. We modeled the interaction of calmodulin with PI3Kα's nSH2 and cSH2 domains, and simulated the calmodulin/cSH2 interaction. The stability of calmodulin/Ca^{2+}/cSH2 interaction was tested with flexible and stiff linker; both bound calmodulin states are stable throughout the simulation (unpublished observation). The nSH2 interaction will be tested as well.

PI3Kα Activation Mechanism

To obtain clues to the structural activation mechanism (108, 112), we superimposed common structural entities in 6 known PI3K crystal structures, and built a structural model of the PI3Kα heterodimer. As depicted in Fig. 4, the model presents all five domains of the p110α catalytic subunit associated with the three p85α nicSH2 (nSH2, iSH2, cSH2) domains of the regulatory subunit. Also included in the structural model are components indispensable for the structural analysis of PI3Kα activation mechanism, including GNP-bound H-Ras bound at RBD, a cosubstrate ATP bound in the cleft between the N- and C- lobes of the catalytic kinase domain, two of the phosphorylated peptides bound, respectively, to nSH2 and cSH2, as well as the head of lipid substrate PIP2 at the entrance of the active site.

As in protein kinases, the lipid kinase activity of PI3Kα is affected by the efficiency of individual steps during the catalytic reaction, including cosubstrate (ATP) binding, substrate (PIP2) binding, phosphoryl transfer and product (ADP and PIP3)

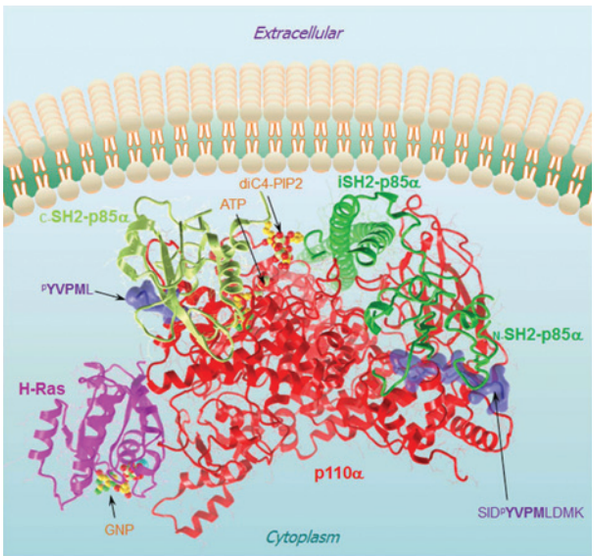

Figure 4.
The structural model of p110α/nicSH2-p85α built from six PDB structures. The PDB codes are 4OVV (p110α/niSH2-p85α/diC4-PIP2), 1E8X (ATP/p110γ), 1HE8 (HRas/p110γ), 1H9O (cSH2-p85α/^{P}YXXM), 2IUI (nSH2-p85α/^{P}YXXM), and 2Y3A (p110β/icSH2-p85). In the model built by simple superimposition-based common structural entities, the active site of kinase domain were bound with ATP and diC4-PIP2. The tail of diC4 chains in PIP2 implicitly points to where the membrane should be. Also included in the constructed complex are the two phosphorylated peptides (with ^{P}YXXM motif) bound respectively to the nSH2 and cSH2 domains as well as an active GNP-bound HRas bound to RBD of p110α.

release. If we assume that both the cosubstrate binding and the product release steps do not play a significant role in PI3Kα activation regulation, then the kinase activation can be assessed by the kinetic measurement of k_a/K_m based on a two-step chemical reaction (113). k_a, the rate constant of the slow phosphate transfer reaction, is inversely proportional to the free energy barrier of the transition state of the phosphate transfer complex. K_m, the equilibrium constant of the fast substrate binding reaction, is inversely proportional to the binding affinity of substrate to PI3Kα.

Studies of cellular (108) as well as oncogenic mutation–elicited PI3Kα activation (114–116) revealed two independent mechanisms. In the first, PI3Kα, an obligate p110α/p85α heterodimer in the cell (117), is activated by the binding of nSH2 domain to the ^{P}YXXM motif of activated RTKs (118) or their associated adaptor proteins (119). The mutually exclusive binding of nSH2-p85α to p110α or to the ^{P}YXXM peptide, as shown in Fig. 4 indicates that the activation is through relieving the autoinhibition of p110α, which is impeded by the regulatory p85α (120). The inhibition role of the nSH2 domain is supported by oncogenic "hot spot" mutations in the helical domain (E542K and E454K) which create same-charge repulsion replacing the highly favorable salt-bridge interactions with the nSH2 domain (121). In the second mechanism, PI3Kα activity is stimulated further by binding of the RBD to Ras-GTP *in vivo* and *in vitro* (122, 123). The allosteric activation triggered by the Ras-GTP binding event seems to exert an effect similar to another oncogenic "hot spot" (H1047R) in the kinase domain (116), causing conformational changes in the C-lobe of the kinase domain located at the membrane interface (121). As

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both events are likely to result in increasing membrane binding to facilitate the accessibility of the kinase domain to the substrate PIP2 on the membrane surface, the H1047R mutant is independent of an interaction with Ras-GTP (116).

In summary, the regulation of PI3Kα activity (124–126) is controlled by two independent mechanisms: PI3Kα membrane–binding capability and the population of effective phosphate transfer transition complexes at the active site. To facilitate the accessibility of the lipid substrate to the active site, evolution has structurally coupled the membrane-binding capability of PI3Kα to its activation, as reflected in the K_m. On the other hand, the release of nSH2-p85α domain from p110α, which relieves the restriction of an effective formation of phosphate transfer transition complex, may dominantly correspond to an increase of k_a. Experimental data indicate that both activation events are required for PI3Kα to achieve a fully active lipid kinase.

Future Prospects

Here we suggest that in PDAC, colorectal cancer, and lung adenocarcinomas, calmodulin/Ca^{2+} can regulate two major pathways, MAPK and PI3K/Akt. Calmodulin/Ca^{2+} temporarily downregulates MAPK; it is required for full activation of PI3Kα by K-Ras4B. GTPase homologs activate PI3K through direct and indirect feedback processes (127); direct interaction of Ras with RBD–p110α is an absolute requirement for *in vivo RAS*-driven tumor formation (123). Endogenous oncogenic K-Ras^{G12V} triggers senescence alone, in the absence of RTK signaling (128). These facts indicate that different from physiologic conditions (68), in cancer a fully activated PI3K pathway is required for cellular growth and proliferation.

This leads us to reason that in adenocarcinomas, cell-specific upregulation of calmodulin/Ca^{2+} expression may substitute for the missing phosphopeptide ^{P}YXXM signal from RTKs. Calmodulin/Ca^{2+} can play two distinct activation roles: as an activator when bound to nSH2-p85α, or as an adaptor when bound to cSH2-p85α. For the first, the prediction of calmodulin/Ca^{2+} interacting with nSH2-p85α by PRISM (105–107) suggests that calmodulin/Ca^{2+} can achieve full PI3Kα activation by relieving the p110α autoinhibition exerted by nSH2-p85α, via a steric hindrance mechanism similar to that induced by the ^{P}YXXM peptide (Fig. 4). For the second, calmodulin has been shown capable of dissociating K-Ras4B, but not its H-Ras or N-Ras isoforms, from membranes in a Ca^{2+}-dependent manner (101), with calmodulin's C-terminal domain binding its farnesylated HVR (72). Our modeling suggests that even when K-Ras4B dissociates from the membrane, calmodulin can fully activate PI3Kα via an allosteric mechanism. PRISM (105–107) models a trimer, K-Ras4B-GTP/calmodulin/PI3Kα (Fig. 3), with an interaction between calmodulin and cSH2-p85α. Calmodulin can act as an adaptor protein to increase the likelihood of K-Ras4B–GTP binding to RBD-p110α (4). In turn, the increase in membrane-binding capability via an enhanced Ras-binding environment allows PI3Kα to remain close to the plasma membrane without relying on K-Ras4B being anchored to membrane. In short, calmodulin can provide the critical missing link in K-Ras4B initiation and progression of pancreatic, colorectal, and lung cancers.

Insight into why and how K-Ras4B can mediate ductal adenocarcinomas, particularly of the pancreas, is vastly significant for adenocarcinoma-specific therapeutics. Here we pointed out the overlooked role of calmodulin in PI3K/Akt signaling. This is based on a wealth of literature and clinical observations and assisted by modeling which shows its feasibility. One way to test our thesis is by experimentally abolishing the K-Ras4B-GTP/calmodulin/PI3Kα trimer in mouse models or oncogenic K-Ras4B ductal cell lines. An inhibitor targeting calmodulin's interaction with p85α cSH2 domain is expected to affect PDAC initiation, cell proliferation, and migration. However, as both MAPK and PI3K/Akt pathways are involved, blocking MAPK signaling is also critical for successful treatment. Our model implies that the K-Ras4B-GTP/calmodulin/PI3K trimer can also serve as an allosteric drug target (129, 130).

Finally, to date calmodulin/K-Ras4B crystallization efforts failed. This could be due to the requirement of farnesylation; it can also reflect the multiple states of calmodulin/K-Ras4B-GTP catalytic domain interactions. Our findings suggest that crystallization efforts may benefit from consideration of a (farnesylated) K-Ras4B-GTP/calmodulin/PI3K trimer.

Here we proposed that Ca^{2+}/calmodulin play a key role in *KRAS*-driven adenocarcinomas by recruiting and activating PI3K at the membrane. We reasoned that calmodulin can act via both PI3Kα/Akt and Raf/MEK/ERK pathways and proposed that a K-Ras4B/calmodulin/PI3Kα trimer could be a propitious adenocarcinoma-specific therapeutic strategy. Our suggestion is in agreement with currently available data; however, ultimately, direct experimental validation is what is needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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