Spatiotemporal Dynamics of Phosphorylation in Lipid Second Messenger Signaling*

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The plasma membrane serves as a dynamic interface that relays information received at the cell surface into the cell. Lipid second messengers coordinate signaling on this platform by recruiting and activating kinases and phosphatases. Specifically, diacylglycerol and phosphatidylinositol 3,4,5-trisphosphate activate protein kinase C and Akt, respectively, which then phosphorylate target proteins to transduce downstream signaling. This review addresses how the spatiotemporal dynamics of protein kinase C and Akt signaling can be monitored using genetically encoded reporters and provides information on how the coordination of signaling at protein scaffolds or membrane microdomains affords fidelity and specificity in phosphorylation events. Molecular & Cellular Proteomics 12: 10.1074/mcp.R113.029819, 3498–3508, 2013.

The alteration of protein or lipid structure by phosphorylation is one of the most effective ways to transduce extracellular signals into cellular actions. Phosphorylation can alter enzyme activity, regulate protein stability, affect protein interactions or localization, or influence other post-translational modifications. A plethora of cellular processes, including cell growth, differentiation, and migration, are tightly regulated by phosphorylation. Cellular homeostasis is achieved by means of a precisely regulated balance between phosphorylation and dephosphorylation, and disruption of this balance results in pathophysiologies. Kinases and phosphatases are antagonizing effector enzymes that respond to second messengers and mediate phosphorylation/dephosphorylation events.

Two prominent lipid second messenger pathways are those mediated by diacylglycerol (DAG)¹ and phosphatidylinositol 3,4,5-trisphosphate (PIP₃) (Fig. 1A). These membrane-embedded second messengers recruit effector kinases containing specific membrane-targeting modules to membranes, thus activating them. Specifically, DAG recruits C1-domain-containing proteins, notably protein kinase C (PKC), whereas PIP₃ recruits pleckstrin homology (PH) domain-containing proteins, such as Akt. Specificity and fidelity in signaling are often achieved via the compartmentalization of signaling on protein scaffolds and membrane microdomains, which can control the access of enzymes to particular substrates. In this review, we provide a brief background of the DAG and PIP₃ pathways and their effector kinases, PKC and Akt, respectively. We discuss sensors that have been developed to measure lipid second messenger levels and kinase activity at various subcellular compartments, the role of scaffolds and membrane microdomains in compartmentalizing signaling, and the consequences of dysregulation of second messenger signaling in disease.

Lipid Second Messengers—Lipid second messengers are signaling molecules produced in response to extracellular stimuli. Targeting enzymes and their substrates to the same membrane constrains them to a space of reduced dimensionality, thus increasing the apparent concentration of the signaling complex and the likelihood and amplitude of signaling (1).

DAG Pathway—Upon activation by agonists, receptor tyrosine kinases and G-protein-coupled receptors can activate phospholipase C, which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP₂) found at the plasma membrane into the second messengers DAG and inositol 1,4,5-trisphosphate (IP₃) (2). DAG recruits proteins that contain C1 domains, small globular DAG-binding domains, to membranes (3), whereas IP₃ freely diffuses inside the cell and binds to the IP₃ receptor at the endoplasmic reticulum. This releases another second messenger, Ca²⁺, which induces further production of DAG at the Golgi (4). Two main classes of proteins that bind DAG are the protein kinases PKC and PKD and the Rac-GAPs chi-merins; the affinity of their C1 domains for DAG can vary greatly and is discussed below in the context of PKC. DAG can also be generated from phosphatidic acid (PA) by phosphatidic acid phosphatases or sphingomyelin synthases. The removal of DAG, and thus termination of its signaling, is achieved by diacylglycerol kinases (DGKs) that convert it into PA (Fig. 1B).

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PIP3 Pathway—The second messenger PIP3 is generated upon the stimulation of receptor tyrosine kinases or G-protein-coupled receptors, which activate phosphoinositide-3 kinase (PI3K), both of which act on phosphatidylinositol 4,5-bisphosphate (PIP2). PI3K converts PIP2 to phosphatidylinositol 3,4,5-trisphosphate (PIP3), which recruits Akt to the plasma membrane via its pleckstrin homology (PH) domain, where it gets activated. PTEN terminates PIP3 signaling by converting it to PIP2. DAG recruited by PIP3 to PI3K can also be converted to DAG and PIP2 by PLC. DAG can then be removed by DGKs as it gets converted to PA. Phosphatidic acid phosphatases (PAPs) and sphingomyelin synthases (SMSs) convert PA back to DAG.

Genetically Encoded Second Messenger Sensors—Second messenger levels can be measured in live cells, in real time, using genetically encoded sensors (6–11). The first generation of sensors comprised a fluorescent protein fused to a domain that specifically binds a second messenger (i.e., PH domain). Recruitment of the tagged domain to membranes containing the second messenger is monitored and used as a proxy for its levels (6, 10, 11). Recently, reporters containing two fluorescent proteins that undergo changes in fluorescence resonance energy transfer (FRET) as they change their distance or relative orientation upon binding of a second messenger have been developed (7–9). These reporters provide more quantitative data, as they rely on ratiometric measurements of two fluorophores as opposed to the translocation of a single fluorophore to membranes, thus minimizing artifacts resulting from cell movements, photobleaching, or variable cell thickness (12). Targeting of these second messenger sensors to particular subcellular compartments provides information on the kinetics and location of second messenger production.

Measuring DAG—One of the first DAG reporters comprised the C1 domain of PKCγ fused to a green fluorescent protein, and its agonist-evoked translocation to membranes served as a readout for DAG production (6). Reporters employing ratiometric measurements were later developed and contain a C1 domain and a FRET pair, such as the DAG reporters DAGR (Fig. 2A), which uses intermolecular FRET (7), and Daglas (Fig. 2B), which uses intramolecular FRET (8). These reporters can be targeted to specific subcellular compartments using a membrane localization sequence, allowing the detection of changes in DAG levels at various intracellular membranes. Such membrane-specific reporters have revealed that the Golgi and endoplasmic reticulum have relatively high basal levels of DAG, whereas the plasma membrane lacks measurable basal DAG (9). DAG at the plasma membrane is produced following acute agonist stimulation, and this signaling is swiftly terminated (Fig. 3) as DGK converts DAG to PA (8, 13). In contrast, stimulated DAG levels at the Golgi are relatively sustained, whereas those at mitochondria are not detectably altered by ATP stimulation.
More recently, a sensor has been engineered to simultaneously measure two second messengers in the same cell. The Green Upward or Downward DAG (Fig. 2C) was paired with a Ca\textsuperscript{2+} sensor (not shown) for the concomitant measurement of DAG and Ca\textsuperscript{2+} (9). Both of these sensors are based on fluorescent proteins that undergo changes in fluorescence intensity upon the binding of second messengers. The DAG sensor (based on PKC\textsuperscript{a}) and the Ca\textsuperscript{2+} sensor (based on calmodulin and a calmodulin-binding domain (14)) are linked by a peptide that, upon cleavage, produces equal amounts of the two reporters as distinct peptides (15). Considering that DAG and Ca\textsuperscript{2+} are often co-elevated, measuring these sec-

ond messengers concomitantly gives a more complete view of signal transduction and has the advantage of providing a direct comparison of second messenger production downstream of the activation of various receptors with different agonists. However, because these reporters rely on changes in fluorescence intensity instead of a FRET ratio, care should be taken when using them, as their readout is dependent on the absolute concentration of the sensor, and intensity changes resulting from focus drift or cell movements can occur during imaging.

Measuring PIP$_3$—Changes in PIP$_3$ levels can be assessed by monitoring the agonist-dependent relocalization of fluorescently tagged PIP$_3$-selective PH domains to membranes (10, 12). However, because these methods monitor translocation to all membranes, pools of PIP$_3$ at specific membranes cannot be readily monitored. Thus, more quantifiable FRET-based PIP$_3$ sensors that can be targeted to particular subcellular localizations have been developed. For example, an indicator for phosphoinositides based on Akt’s PH domain, InPAkt (Fig. 2D), showed that the plasma membrane contains basal levels of PIP$_3$ that are maintained through a balance between PI3K and phosphatases, whereas the nucleus has no detectible production of this second messenger (16). Another PIP$_3$ sensor, Fllip (Fig. 2B), comprising the PH domain of GRP1 and a FRET pair, is anchored to a membrane via a membrane localization sequence (17). This reporter revealed that platelet-derived growth factor (PDGF) stimulates greater production of PIP$_3$ at the Golgi and endoplasmic reticulum than at the plasma membrane, and that PIP$_3$ is generated at these endomembranes by endocytosed receptor tyrosine kinases that activate a localized pool of PI3K.

PKC and Akt as Effectors—PKC and Akt, effector kinases of DAG and PIP$_3$, respectively, phosphorylate myriad downstream targets (reviewed in Refs. 18–20), many of which have been identified through phosphoproteomic screens (21–23).

PKC—The PKC family consists of nine genes that are divided into three categories based on the domain structure of the enzymes they encode, and hence the second messengers they require for activation. Both conventional PKCs (cPKCs) (α, β, γ) and novel PKCs (nPKCs) (δ, ε, η, ι, ζ, ξ, Ω) bind DAG through tandem C1 domains; cPKCs also bind membranes in a Ca$^{2+}$-dependent manner through a C2 domain. Atypical PKCs

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**Fig. 3.** Schematic diagram displaying DAG and PIP$_3$ levels at various intracellular membranes and the PKC and Akt activity they induce at these locations. UTP stimulates rapid but short-lived production of DAG at the plasma membrane. In contrast, stimulated DAG levels at the Golgi are relatively sustained, leading to more sustained PKC activity at the Golgi than at the plasma membrane. Typically cPKCs are recruited to the plasma membrane because their Ca$^{2+}$-regulated C2 domain selectively recognizes PIP$_3$, which is enriched at the plasma membrane. In contrast, nPKCs signal primarily at the DAG-enriched Golgi (111). The nucleus shows little UTP-induced PKC activity, whereas the cytosol has the greatest, although transient, UTP-stimulated PKC activity. Upon PDGF stimulation, PIP$_3$ is more rapidly generated at endomembranes such as the Golgi than at the plasma membrane. PDGF-induced Akt activity rapidly increases at the plasma membrane, whereas in the cytosol the kinetics of activation are slower. The nucleus contains high but delayed Akt activity, despite the lack of PIP$_3$ production at the nuclear membrane, suggesting that active Akt translocates to the nucleus where there is little phosphatase suppression.
Measuring Localized PKC and Akt Signaling

(ζ and η/λ) bind neither DAG nor Ca\(^{2+}\) and are regulated by protein–protein interactions through a PB1 domain (24). cPKCs and nPKCs are constitutively phosphorylated at three conserved phosphorylation sites termed the activation loop, turn motif, and hydrophobic motif (25). These phosphorylations are necessary for proper PKC folding, and thus for its activation; for PKCα, these modifications occur with a halftime of 5 to 10 min following biosynthesis (26). The activation loop is phosphorylated by the phosphoinositide-dependent kinase PDK-1 (27–29), an event that triggers two tightly coupled phosphorylations at the turn and hydrophobic motifs (25, 30). mTORC2 is required to initiate the phosphorylation cascade, and in cells lacking mTORC2, PKC is not phosphorylated and thus is shunted for degradation (31–33); however, the mechanism of this regulation is unknown. The hydrophobic motif is autophosphorylated by an intramolecular reaction in vitro, but whether this is the mechanism of modification in cells or whether it is the direct target of another kinase such as mTORC2 remains controversial (33, 34). When first translated, PKC is in an open conformation, with the autoinhibitory pseudosubstrate out of the active site (35). Upon phosphorylation, PKC matures into a catalytically competent, but inactive, species that is maintained in an autoinhibited (closed) conformation in which the pseudosubstrate occupies the substrate-binding cavity. Upon intracellular Ca\(^{2+}\) release and DAG production, cPKCs are recruited to membranes through their Ca\(^{2+}\)-sensitive C2 domain. This relocalization reduces the dimensionality in which the C1 domain has to probe for its membrane-embedded ligand, DAG, thus increasing the effectiveness of this search by several orders of magnitude (36). Binding of one of PKC’s C1 domains to DAG provides the energy necessary to expel the pseudosubstrate from the substrate-binding site, allowing the phosphorylation of downstream targets. For some isoforms, such as PKCθ, the second C1 domain (C1B) is the major binder (37). The affinity of C1 domains for DAG is toggled from low to high by a single residue within the DAG binding cavity: a Trp at position 22 confers an affinity for DAG that is 2 orders of magnitude higher than that conferred by a Tyr at that position (38). Consequently, nPKCs, which contain a high-affinity C1B domain, can respond to DAG alone, whereas cPKCs, which have a low-affinity C1B domain, require the elevation of Ca\(^{2+}\) concurrently with DAG production in order to become activated. Reporters such as the Green Upward or Downward DAG (Fig. 2C) paired with a Ca\(^{2+}\) sensor (9), described above, would be useful tools for discerning which agonists solely activate cPKCs versus nPKCs.

The amplitude of PKC signaling is diligently balanced through its phosphorylation state (controlling its steady-state levels), the presence of its lipid second messenger, DAG (acutely controlling activity), and, for cPKCs, Ca\(^{2+}\) levels. Thus PKC activity can be antagonized via direct dephosphorylation by protein phosphatases such as the PH domain leucine-rich repeat protein phosphatase (PILPP) or by removal of the lipid second messenger through phosphorylation by the lipid kinase DGK (Fig. 1) (39). The peptidyl-prolyl isomerase Pin1 was recently shown to be necessary for cPKC dephosphorylation and degradation following agonist activation, as it isomerizes the phosphorylated turn motif of cPKCs, thus facilitating PKC dephosphorylation (40).

Akt—Akt, also known as PKB because of its homology to PKA and PKC, is a serine/threonine kinase that promotes cell growth and survival (41). The three Akt isoforms (Akt1, Akt2, and Akt3) contain an N-terminal PH domain that mediates PIP3-dependent membrane recruitment. Akt is maintained in an inactive conformation by an interaction between the PH and kinase domains, and unlike PKC, Akt is directly activated by phosphorylation at its activation loop and hydrophobic motif following agonist-dependent recruitment to membranes (42). Thus, Akt phosphorylation at these sites can be used as a proxy for activity under certain conditions (43). Akt is co-translationally phosphorylated at the turn motif by mTORC2 (contrasting with the post-translational modification of PKC), an event that is not necessary for function but increases the stability of the protein (44). PIP3 recruits Akt to the plasma membrane via its PH domain, unmasking the kinase domain to permit phosphorylation of the activation loop by PDK-1 (45) and subsequent phosphorylation of the hydrophobic motif. mTORC2 facilitates hydrophobic motif phosphorylation of Akt (46, 47), possibly by assisting in unmasking the kinase domain for phosphorylation. Indeed, manipulations that displace the PH domain effectively bypass the requirement for mTORC2 for phosphorylation of the hydrophobic motif (but not the turn motif) (48). Phosphorylated Akt is locked in an active conformation and can disengage from the membrane and relocalize to other intracellular regions, such as the nucleus (49), to phosphorylate diverse substrates (19). Akt signaling is terminated by the hydrolysis of PIP3 to PIP2 by PTEN or by direct dephosphorylation of the activation loop by protein phosphatase 2A or of the hydrophobic motif by PHLPP (50–52). PHLPP1 dephosphorylates Akt2 and Akt3, whereas PHLPP2 dephosphorylates Akt1 and Akt3 (52), suggesting that these isoforms are differentially compartmentalized, likely via the unique PDZ (PSD-95, disheveled, and ZO1) ligand of each PHLPP isozyme (53). Interestingly, stoichiometric quantification of Akt phosphorylation at the activation loop and hydrophobic motif sites using LC-MS revealed that in untreated T cells, less than 1% of Akt is phosphorylated at both of these sites, and a low level of Akt phosphorylation is sufficient to contribute to tumorigenesis (54), highlighting the importance of keeping Akt activity low for maintaining cellular homeostasis.

Identifying PKC and Akt Substrates—A number of PKC and Akt substrates have been identified through biochemical methods, and several phosphoproteomic screens have been devised to identify new substrates and novel roles of these kinases. For example, a functional proteomic screen identified enhancer of mRNA decapping 3 as a substrate of Akt that...
regulates the mRNA decay and translation repression pathways downstream of insulin signaling (21), and another identified a number of chaperone proteins and protein disulfide isomerases as potential Akt substrates in rat mesangial cells, suggesting that Akt might regulate chaperone function (22). An in vivo quantitative phosphoproteomics study employing stable isotope labeling by amino acids in cell culture implicated a highly deregulated kinase network composed of PKC, PAK4, and SRC in squamous cell carcinoma, but not in papilloma (55).

Vast efforts combining phosphorylation sites identified in vivo though mass spectrometry, in vitro through protein microarrays, and computationally through prediction algorithms have uncovered more substrates, setting the foundation for the development of human phosphorylation network maps (23, 56). However, despite efforts to enrich for physiologically relevant phosphorylation events by accounting for subcellular localization and scaffolding, these networks still have shortcomings, as the algorithms predict some false positives and miss a large fraction of known phosphorylation sites.

Kinase Activity Reporters—Genetically encoded reporters allow the visualization of the spatiotemporal dynamics of kinase activity in individual cells. These reporters can be targeted to various subcellular localizations and to protein scaffolds to measure localized activity, which can be more physiologically relevant than bulk activity in the cytosol.

PKC—Because cPKCs and nPKCs are constitutively phosphorylated at the C-terminal sites, and because the phosphate at the activation loop does not modulate activity once the C-terminal tail is phosphorylated (25), their activity cannot be measured with phosphorylation-specific antibodies, as is done for most other kinases. However, PKC activity can be monitored using activity reporters such as the C kinase activity reporter (Fig. 2E), which is composed of a CFP–YFP FRET pair flanking a PKC-specific substrate and an FHA2 phosphothreonine-binding domain (7). Upon phosphorylation of this substrate by PKC, the reporter undergoes a conformational change that decreases FRET. As phosphorylation of the reporter is reversible (i.e. phosphatases can dephosphorylate the reporter), it provides a real-time readout of PKC activity.

C kinase activity reporter has been targeted to various intracellular locations to enable specific monitoring of PKC activity at these regions. Using these targeted reporters, Gallegos et al. (13) found that the activation of PKC with the agonist UTP leads to rapid and relatively sustained PKC activity at the Golgi, driven by the persistence of DAG at this membrane (Fig. 3). UTP-dependent PKC activity in the cytosol is, however, quickly terminated by phosphatases, and activity in the nucleus is low because of high phosphatase suppression in this compartment. The mitochondria also have little UTP-stimulated activity; however, using a mitochondrially targeted PKCα-specific activity reporter, Mito-ΔCKAR, Zheng et al. (57) revealed that PKCα translocates to, and is active at, the outer membrane of mitochondria upon stimulation with phorbol esters, and that its intrinsic catalytic activity is required for its interaction with the mitochondria.

The Schultz lab has developed a reporter for nPKCs and atypical PKCs, KCP-1 (Fig. 2F), that is based on the PKC substrate pleckstrin (58). This reporter does not utilize a phosphopeptide-binding domain; rather, phosphorylation of residues between its PH and dishelved-Egl-10–pleckstrin domains causes a conformational change in the reporter, resulting in a change in FRET. Thus, interactions between the phosphorylated sites and other endogenous proteins are reduced.

Akt—A number of FRET-based Akt reporters have been developed, most of which measure the phosphorylation of a synthetic substrate of Akt in live cells; these include Aktus, AktAR (Fig. 2G), and BKAR (Fig. 2E) (59–61). Aktus has low sensitivity and requires overexpression of Akt, whereas BKAR and AktAR can sense endogenous Akt activity. Because activated Akt can disengage from the membrane and diffuse to other subcellular locations, targeting these reporters to various subcellular compartments is particularly useful. BKAR targeted to the plasma membrane revealed that phosphatase suppression of Akt is low at this location (60). The phosphatase suppression of Akt activity is greater in the cytosol than it is at the plasma membrane, so Akt is more rapidly inactivated in the cytosol (Fig. 3), most likely by protein phosphatases that dephosphorylate Akt’s substrates as opposed to Akt itself. Conversely, the nucleus has low phosphatase suppression of Akt, so once Akt diffuses to the nucleus its activity is much more sustained (60). AktAR (61) has a greater dynamic range for detecting Akt activity than BKAR and was used to measure Akt activity in plasma membrane microdomains (addressed below).

Whereas fluorescent reporters can measure rapid signaling kinetics at subcellular levels, bioluminescent reporters have the advantage of producing their own light and therefore bypass issues with autofluorescence, photobleaching, and tissue damage from the excitation light (62). A bioluminescent Akt reporter, BAR, was engineered using a split luciferase, an Akt specific substrate, and a phosphopeptide-binding domain (Fig. 2H). This reporter has the capability to measure Akt activity in a noninvasive manner in vivo (63). In addition to Akt activity reporters that measure the phosphorylation of a synthetic substrate by Akt, reporters that measure conformational changes of Akt induced by its translocation and phosphorylation (and thus activity state) have also been developed (64–66). The Akt indicator Akind (57), comprising the PH and catalytic domains of Akt and a FRET pair (Fig. 2I), was used to visualize Akt translocation to and activity at lamellipodial protrusions. Conformational changes attendant to the phosphorylation of Akt result in an increase in FRET. The use of another reporter of Akt action, ReAktion (not shown) (58), led to the proposal that activation loop phosphorylation of Akt decreases its membrane binding affinity, thus allowing disengagement from the membrane and relocation to other
Localized Signaling and the Importance of Scaffolding—Protein scaffolds coordinate and allow specificity and fidelity by compartmentalizing kinases and their downstream substrates, as well as the phosphatases that can rapidly terminate the signal (67). Even though lipid second messengers acutely regulate the activity of PKA and Akt, scaffolds can mediate their access to particular substrates.

PKC Scaffolds—Although multiple PKC isozymes respond to the same second messengers, there is some specificity in their function and signaling, mediated in part by their cell-specific pattern of expression, their differential affinities for certain lipids, and by protein scaffolds. PKC is anchored to numerous protein scaffolds through interactions mediated by its regulatory domain, its pseudosubstrate, or, in the case of PKCα and the atypical PKCs, a PDZ ligand. The first PKC scaffolds were identified by Mochly-Rosen and colleagues as receptors for activated C kinase, which are proposed to selectively bind active PKC and enhance its activity toward substrates anchored at that location (68, 69). A kinase anchoring proteins (AKAPs), which were first identified as PKA scaffolds (70), also anchor PKC in proximity to its targets, but in its inactive state, thus enabling rapid downstream signaling upon PKC activation. For example, AKAP-Lbc coordinates PKCγ and PKA to phosphorylate PKD and release it from the scaffold (71), whereas AKAP79 functions as a scaffold for PKC, PKA, and protein phosphatase 2B at the postsynaptic density in neurons (72). One critical aspect of scaffolding is that it can alter the pharmacological profile of tethered kinases, which could have clinical implications for drug design. For example, through the use of targeted PKC activity reporters, Scott and colleagues found that PKC bound to the AKAP79 scaffold is refractory to active site inhibitors of the co-scaffolded PDK-1 (73).

PKCα, -ζ, and -ε also bind PDZ-domain-containing scaffolds through their distinct PDZ ligands. In the case of PKCα, scaffolding by its PDZ ligand is required for cerebellar long-term depression (74). One likely PDZ-domain-binding partner involved in this is protein interacting with Ca2+ kinase (PIPK1), which interacts specifically with the PDZ ligand of PKCα (75, 76). Interestingly, PIPK1 can have opposing effects on PKCα function in neurons, where it can act as either a mediator or an inhibitor of the phosphorylation of downstream targets. PIPK1 targets activated PKCα to synapses to phosphorylate the glutamate receptor subunit GluR2, leading to its endocytosis (77), but it can also act as a barrier to phosphorylation of the metabotropic glutamate receptor mGluR7a by PKCα (78). More recently, a family of Discs large homolog scaffolds that interact with the PDZ ligand of PKCα to facilitate cellular migration was also identified (79).

Akt Scaffolds—Akt translocation to different intracellular regions is contingent on which upstream pathway is activated, partially because of the scaffolding proteins that direct its signaling. For example, the stimulation of endothelial cells with insulin leads to the activation of Akt and its translocation to both the Golgi and mitochondria, whereas stimulation with 17β-estradiol leads to translocation of Akt to the Golgi but not mitochondria (59). Only a few Akt scaffolds have been identified thus far, but it is becoming more apparent that different receptors use different complexes to direct Akt activity (80). For example, Akt kinase-interacting protein 1 was identified as a scaffold for PI3K/PDK-1/Akt that associates with activated EGF receptors (81). This scaffold is necessary for Akt phosphorylation by PDK-1 downstream of EGF signaling. Conversely, Akt scaffolds can also attenuate Akt signaling by scaffolding it in proximity to its phosphatases. β-arrestin 2 scaffolds Akt and its activation loop phosphatase, PP2A, in response to G-protein-coupled receptor stimulation in dopaminergic neurotransmission (82), and β-arrestin 1 scaffolds Akt1 and its hydrophobic motif phosphatase, PHLPP2, downstream of receptor tyrosine kinases (83). Considering that the three Akt isozymes have some overlapping expression, are activated downstream of a multitude of receptors, have numerous cellular functions, and show specificity in their dephosphorylation by PHLPP, an abundance of Akt scaffolds potentially await identification.

Membrane Microdomains—Lipid rafts, which are cholesterol- and sphingolipid-rich microdomains, not only compartmentalize signaling complexes, but also increase signal transduction by aggregating particular signaling complexes in a small area (1).

PKC—DAG and its effector kinase PKC are often enriched in specialized lipid rafts that form membrane invaginations called caveolae (84, 85). For example, activation of the adenosine A1 receptor in cardiomyocytes causes PKCζ and PKCδ to translocate to caveolae. PKCα is also enriched within caveolae through interactions with cavin1, which inhibits its activity, or with the serum deprivation response protein (86, 87).

Akt—Akt signaling not only differs at various subcellular localizations, but also varies within microdomains of a particular membrane. The Akt reporter AktAR (Fig. 2G) was preferentially targeted to different plasma membrane microdomains such as lipid rafts (using the N-terminal region of Lyn kinase) and non-raft regions (using the Kras CAAX motif) to analyze the spatiotemporal dynamics of Akt activity (61). These reporters demonstrate that Akt residing in lipid rafts is activated more potently and with faster kinetics than non-raft Akt. PDK-1 is enriched in membrane microdomains, partially accounting for the increased Akt activity in this compartment (88, 89).
Membrane microdomains provide sheltered environments in which signaling is protected from immediate termination. For example, PI3K gets activated and produces PIP3 in lipid rafts where Akt is recruited, enabling Akt to be rapidly and specifically activated by PDK-1 (90). However, PTEN, which terminates PIP3 signaling, is primarily found in non-raft microdomains, allowing PIP3 levels to be temporarily maintained in lipid rafts, underscoring the importance of spatially separating kinases from phosphatases. Disturbing this spatial segregation of negative and positive regulators of a pathway can lead to disease states.

**Dysregulation of Lipid Signaling in Disease**—Dysregulation of the PIP3 and DAG signaling pathways leads to numerous pathophysiologies, such as inflammation, cardiovascular disease, diabetes, neurodegeneration, and cancer (91). Imbalances in these pathways are caused by mutations, gene amplifications or deletions, chromosomal translocations, or epigenetic changes of genes in these pathways. In general, enzymes that promote signaling such as PKC, Akt, and the catalytic subunit of PI3K have been identified as oncogenes (92–94), whereas those that terminate signaling—PTEN, PHLPP, and DGK—have been implicated as tumor suppressors (53, 95–100).

Although mutations in Akt are uncommon, Akt signaling is often elevated in cancer. Indeed, the PI3K pathway is one of the most frequently up-regulated pathways in cancer (101–103). Mutations that inactivate PTEN or hyperactivate the catalytic subunit of PI3K are very common and induce constitutive Akt signaling. Interestingly, a functional proteomics study using a reverse-phase protein lysate array revealed that in breast cancers, Akt activation loop and hydrophobic motif phosphorylation strongly inversely correlates with PTEN levels, underscoring the importance of PTEN regulation of Akt activity (104).

Mutations in receptors upstream of DAG and PIP3 can also lead to enhanced signaling. For example, certain EGF receptor mutants constitutively associate with the PI3K/PDK-1/Akt scaffold Akt kinase-interacting protein 1, leading to increased Akt signaling in lung cancer (105). The involvement of some of these genes in cancer is further substantiated by their frequent amplification or deletion in tumors. For example, genes encoding PKC, Akt1, Akt2, and the catalytic subunit of PI3K are amplified in various cancers (92, 94, 106–109), whereas those encoding PTEN and PHLPP are commonly deleted (110).

**CONCLUSIONS**

Extracellular stimuli lead to the rapid but transient production of localized pools of DAG and PIP3 at discrete subcellular locations. The kinetics of the production and removal of these second messengers vary at different intracellular regions, leading to unique signaling signatures at each location. Protein scaffolds and membrane microdomains play key roles in signaling by providing ways to mediate the action of promiscuous enzymes. As we enter the frontier of localized signaling, reporters that measure lipid second messenger levels and kinase activity at the diverse signaling platforms afforded by intracellular membranes, membrane microdomains, and scaffolds will become increasingly sought after as a means to precisely measure activity downstream of particular ligands and receptors. Moreover, our knowledge of the human phosphorylation network will continue to expand, as kinase–substrate relationships are systematically being tackled.

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**REFERENCES**

1. Kholodenko, B. N., Hoek, J. B., and Westerhoff, H. V. (2000) Why cytoplasmic signaling proteins should be recruited to cell membranes. Trends Cell Biol. 10, 173–178
2. Berridge, M. J. (1987) Inositol trisphosphate and diacylglycerol: two interacting second messengers. Annu. Rev. Biochem. 56, 159–193
3. Sharkey, N. A., Leach, K. L., and Blumberg, P. M. (1984) Competitive inhibition by diacylglycerol of specific phorbol ester binding. Proc. Natl. Acad. Sci. U.S.A. 81, 607–610
4. Kunkel, M. T., and Newton, A. C. (2010) Calcium transduces plasma membrane receptor signals to produce diacylglycerol at Golgi membranes. J. Biol. Chem. 285, 22748–22752
5. Yu, J. W., Mendrola, J. M., Audhya, A., Singh, S., Keleti, D., DeWald, D. B., Murray, D., Emr, S. D., and Lemmon, M. A. (2004) Genome-wide analysis of membrane targeting by S. cerevisiae pleckstrin homology domains. Mol. Cell. 13, 677–688
6. Oancea, E., Tereul, M. N., Quest, A. F., and Meyer, T. (1998) Green fluorescent protein (GFP)-tagged cysteine-rich domains from protein kinase C as fluorescent indicators for diacylglycerol signaling in living cells. J. Cell Biol. 140, 485–498
7. Violin, J. D., Zhang, J., Tsien, R. Y., and Newton, A. C. (2003) A genetically encoded fluorescent reporter reveals oscillatory phosphorylation by protein kinase C. J. Cell Biol. 161, 899–909
8. Sato, M., Ueda, Y., and Umewaza, Y. (2006) Imaging diacylglycerol dynamics at organellar membranes. Nat. Methods 3, 797–799
9. Tevson, P., Westenberg, M., Zhao, Y., Campbell, R. E., Quinn, A. M., and Hughes, T. E. (2012) Simultaneous detection of Ca2+ and diacylglycerol signaling in living cells. PLoS One 7, e42791
10. Watton, S. J., and Downward, J. (1999) Akt/PKB localisation and 3-phosphoinositide generation at sites of epithelial cell-matrix and cell-cell interaction. Curr. Biol. 9, 433–436
11. Venkateswarlu, K., Gunn-Moore, F., Oatley, P. B., Tavare, J. M., and Cullen, P. J. (1998) Nerve growth factor- and epidermal growth factor-stimulated translocation of the ADP-ribosylation factor-exchange factor GRP1 to the plasma membrane of PC12 cells requires activation of phosphatidylinositol 3-kinase and the GRP1 pleckstrin homology domain. Biochem. J. 335(Pt 1), 139–146
12. Halet, G. (2005) Imaging phosphoinositide dynamics using GFP-tagged protein domains. Biol. Cell. 97, 501–518
13. Gallegos, L. L., Kunkel, M. T., and Newton, A. C. (2006) Targeting protein kinase C activity reporter to discrete intracellular regions reveals spatiotemporal differences in agonist-dependent signaling. J. Biol. Chem. 281, 30947–30956
14. Zhao, Y., Araki, S., Wu, J., Teramoto, T., Chang, Y. F., Nakano, M.,
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Abdelfattah, A. S., Fujiiwa, M., Ishihara, T., Nagai, T., and Campbell, R. E. (2011) An expanded palette of genetically encoded Ca(2+){\(\rightarrow\)} indicators. Science 333, 1888–1891

15. Szymczak, A. L., Workman, C. J., Wang, Y., Vignali, K. M., Diliglou, S., Vanin, E. F., and Vignali, D. A. (2004) Correction of multi-genic deficiency in vivo using a single “self-cleaving” 2A peptide-based retroviral vector. Nat. Biotechnol. 22, 589–594

16. Ananthanarayanan, B., Ni, Q., and Zhang, J. (2005) Signal propagation from membrane messengers to nuclear effectors revealed by reporters of phosphoinositide dynamics and Akt activity. Proc. Natl. Acad. Sci. U.S.A. 102, 15081–15086

17. Sato, M., Ueda, Y., Takagi, T., and Umezawa, Y. (2003) Production of PtdInsP3 at endomembranes is triggered by receptor endocytosis. Nat. Cell Biol. 5, 1016–1022

18. Rinck, M., Kermorgant, S., Camarero, A. J., Boeczekler, K., and Parker, P. J. (2010) PKC and the control of localized signal dynamics. Nat. Rev. Mol. Cell Biol. 11, 103–112

19. Manning, B. D., and Cantley, L. C. (2007) AKT/PKB signaling: navigating downstream. Cell 129, 1261–1274

20. Griner, E. M., and Kazanietz, M. G. (2007) Protein kinase C and other diacylglycerol effectors in cancer. Nat. Rev. Cancer 7, 281–294

21. Larner, J. M., Rowland, A. F., Hoehn, K. L., Humphreys, D. T., Preiss, T., Guilhaus, M., and James, D. E. (2010) Global phosphoproteomics identifies a major role for Akt-14–3-3 in regulating EDC3. Mol. Cell. Proteomics 9, 682–694

22. Barati, M. T., Rane, M. J., Klein, J. B., and McLeish, K. R. (2006) A proteomic screen identified stress-induced chaperone proteins as targets of Akt phosphorylation in mesangial cells. J. Proteome Res. 5, 1042–1045

23. Newman, R. H., Hu, J., Rhoe, H. S., Xie, Z., Woodard, C., Neiswinger, J., Cooper, C., Shirley, M., Clark, H. M., Hu, S., Hwang, W., Seop Jeong, J., Blackshaw, S., Pawson, T., Gingras, A. C., Desiderio, S., Pandey, A., Blackshaw, S., Roux, C., Xia, S., Ji, H., Dalby, K. N., Turk, B. E., Zhang, J. H., and Qian, J. (2013) Construction of human activity-based phosphorylation networks. Mol. Syst. Biol. 9, 655

24. Lenoir, T., Parent, M., Quetsen, H., Kristiansen, K., Overvatn, A., Michaelsen, E., Bjorkoy, G., and Johansen, T. (2003) Interaction codes within the family of mammalian Phox and Bem1p domain-containing proteins. J. Biol. Chem. 278, 34568–34581

25. Keranen, L. M., Dutli, E. M., and Newton, A. C. (1995) Protein kinase C is regulated in vivo by three functionally distinct phosphorylations. Curr. Biol. 5, 1391–1400

26. Sonntag, F. P. E., D. G., Tao, T., and Newton, A. C. (2001) The phosphoinositide-dependent kinase, PDK-1, phosphorylates conventional protein kinase C isoforms by a mechanism that is independent of phosphoinositide 3-kinase. J. Biol. Chem. 276, 45289–45297

27. Le Good, J. A., Ziegler, W. H., Parekh, D. B., Alessi, D. R., Cohen, P., and Newton, A. C. (1998) Regulation of conventional protein kinase C isozymes. J. Biol. Chem. 273, 29359–29362

28. Dries, D. R., Gallegos, L. L., and Newton, A. C. (2007) A single residue in the C1 domain sensitizes novel protein kinase C isoforms to cellular diacylglycerol production. J. Biol. Chem. 282, 862–830

29. Gao, T., Brognard, J., and Newton, A. C. (2008) The phosphatase PHLPP controls the cellular levels of protein kinase C. J. Biol. Chem. 283, 6300–6311

30. Nalefski, E. A., O'Neill, A. K., Kannan, K., Kruse, N., Taylor, S. S., Jennings, P. A., and Newton, A. C. (2012) Peptidyl-prolyl isomerase Pin1 controls down-regulation of conventional protein kinase C isoforms. J. Biol. Chem. 287, 13262–13278

31. Tilton, B., Andjelkovic, M., Didichenko, S. A., Hemmings, B. A., and Thelen, M. (1997) G(1)-protein-coupled receptors and G-coupled-receptors mediate activation of Akt/protein kinase B in human phagocytes. J. Biol. Chem. 272, 28206–28211

32. Calleja, V., Alcor, D., Laguerre, M., Park, J., Voinovich, B., Hemmings, B. A., Downward, J., Parker, P. J., and Larijani, B. (2007) Intramolecular and intermolecular interactions of protein kinase B define its activation in vivo. PLoS Biol. 5, e95

33. Kumar, N., Ayefan, R., Sheppard, S., Harms, B., and Lauffenburger, D. A. (2007) Quantitative analysis of Akt phosphorylation and activity in response to EGF and insulin treatment. Biochem. Biophys. Res. Commun. 354, 14–20

34. Oh, W. J., Wu, C. C., Kim, S. J., Facchinetti, V., Julien, L. A., Finlan, M., Roux, P. P., Su, B., and Jacinto, E. (2010) mTORC2 can associate with ribosomes to promote cotranslational phosphorylation and stability of nascent Akt polypeptide. EMBO J. 29, 3939–3951

35. Alesi, D. R., James, S. R., Downes, C. P., Holmes, A. B., Gaffney, P. R., Reese, C. B., and Cohen, P. (1997) Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase B alpha. Cell. 7, 261–269

36. Sarbassov, D. D., Guerin, D. A., Ali, S. M., and Sabatini, D. M. (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307, 1098–1101

37. Toker, A., and Newton, A. C. (2000) Akt/protein kinase B is regulated by autophosphorylation at the hypothetical PKD-2 site. J. Biol. Chem. 275, 8271–8274

38. Warfel, N. A., Niederst, M., and Newton, A. C. (2011) Disruption of the interface between the pleckstrin homology (PH) and kinase domains of Akt protein is sufficient for hydrophobic motif site phosphorylation in the absence of mTORC2. J. Biol. Chem. 286, 39122–39129

39. Meier, R., Alesi, D. R., Cron, P., Andjelkovic, M., and Hemmings, B. A. (1997) Mitogogenic activation, phosphorylation, and nuclear translocation of protein kinase B beta. J. Biol. Chem. 272, 30491–30497

40. Andjelkovic, M., Jakubowicz, T., Cron, P., Ming, X. F., Han, J. W., and Hemmings, B. A. (1996) Activation and phosphorylation of a pleckstrin homology domain containing protein kinase (RAC-PK/PKB) promoted by serum and protein phoshatase inhibitors. Proc. Natl. Acad. Sci. U.S.A. 93, 5699–5704

41. Stambolic, V., Suzuki, A., de la Pompa, J. L., Brothers, G. M., Mirtsos, C., Sasaki, T., Ruland, J., Penninger, J. M., Siderovski, D. P., and Mack, T. W. (1998) Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell 95, 29–39

42. Brognard, J., Sieriecki, E., Gao, T., and Newton, A. C. (2007) PHLP2 and a second isoform, PHLP2P2, differentially attenuate the amplitude of Akt signaling by regulating distinct Akt isoforms. Mol. Cell. 25, 917–931

43. Gao, T., Furnari, F., and Newton, A. C. (2005) PHLP: a phosphatase that...
directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. Mol. Cell, 18, 13–24

54. Athir, A., Turnock, D., Sellar, G., Thompson, A., Feuerstein, G., Ferguson, M. A., and Huang, J. T. (2010) Stoichiometric quantification of Akt phosphorylation using LC-MS/MS. J. Proteome Res., 9, 743–751

55. Zanivan, S., Meves, A., Behrendt, K., Schoof, E. M., Neillon, L. J., Cox, J., Tang, H. R., Kalina, G., van Rees, J. H., van Deursen, J. M., Trempus, C. S., Machesky, L. M., Linding, R., Wickstrom, S. A., Fassler, R., and Mann, M. (2013) In vivo SILAC-based proteomics reveals phosphoproteome changes during mouse skin carcinogenesis. Cell Rep., 3, 552–566

56. Linding, R., Jensen, L. J., Ostheimer, G. J., van Vugt, M. A., Jorgensen, C., Zehra, S., Bylund, P. A., and Newton, A. C. (2005) In vivo imaging of PKC activity by the caveolin scaffolding domain peptide. J. Biol. Chem., 280, 11766–11773

57. Zheng, Y., Zhang, L., Jia, X., Wang, H., and Hu, Y. (2012) Interaction of protein inhibitor of activated STAT 2 (PIAS2) with receptor of activated C kinase 1, RACK1. FEBS Lett., 586, 122–126

58. Schleifenbaum, A., Stier, G., Gasch, A., Sattler, M., and Schultz, C. (2004) Genetically encoded FRET probe for PKC activity based on pleckstrin. J. Biol. Chem., 279, 11786–11787

59. Sasaki, K., Sato, M., and Umegawa, Y. (2003) Fluorescent indicators for Akt/protein kinase B and dynamics of Akt activity visualized in living cells. J. Biol. Chem., 278, 30945–30951

60. Kunkel, M. T., Ni, Q., Tsien, R. Y., Zhang, J., and Newton, A. C. (2005) Spatio-temporal dynamics of protein kinase B/Akt signaling revealed by a genetically encoded fluorescent reporter. J. Biol. Chem., 280, 5683–5687

61. Gao, X., and Zhang, J. (2008) Spatiotemporal analysis of differential Akt regulation in plasma membrane microdomains. Mol. Biol. Cell, 19, 4366–4373

62. Okumoto, S., Jones, A., and Frommer, W. B. (2012) Quantitative imaging of Akt/protein kinase B and dynamics of Akt activity visualized in living cells. J. Cell Biol., 201, 363–364

63. Galluzzi, L., Vacher-Beg, M. S., Vojnovic, B. Woscholski, R., Downward, J., and Larijani, B. (2003) Monitoring conformational changes of proteins in cells by fluorescence lifetime imaging microscopy. Biochem. J., 372, 33–40

64. Scott, J. D., and Newton, A. C. (2012) Shedding light on local kinase activation. BMC Biol., 10, 61

65. Ron, D., Chen, C. H., Caldwell, J., Jamieson, L., Orr, E., and Mochly-Rosen, D. (1994) Cloning of an intracellular receptor for protein kinase C isoforms to the caveolin-rich plasma membrane microdomain. J. Biol. Chem., 269, 25512–25516

66. Klauck, T. M., Faux, M. C., Labudda, K., Langeberg, L. K., Jaken, S., and Scott, J. D. (2006) Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. Science, 271, 1599–1602

67. Horsch, A. H., Glantz, S. B., Li, Y., You, Y., and Rubin, C. S. (1992) Cloning and expression of an intron-less gene for AKAP75, an anchor protein for the regulatory subunit of cAMP-dependent protein kinase II beta. J. Biol. Chem., 267, 2131–2134

68. Lord, P. W., Neves, P. A., Zoncu, R., Mora, M., and Scott, J. D. (2010) Interaction with AKAP79 modifies the cellular pharmacology of PKC. Mol. Cell, 37, 541–550

69. Leitges, M., Kovic, J., Plomann, M., and Linden, D. J. (2004) A unique PDZ ligand in PKC alpha confers induction of cerebellar long-term synaptic depression. Neuron, 44, 585–594

70. Steudinger, J., Zhuo, Z., Burgess, R., Elledge, S. J., and Olson, E. N. (1995) PICK1: a perinuclear binding protein and substrate for protein kinase C isolated by the yeast two-hybrid system. J. Cell Biol., 128, 263–271

71. Steudinger, J., Lu, J., and Olson, E. N. (1997) Specific interaction of the PDZ domain protein PICK1 with the COOH terminus of protein kinase C-alpha. J. Biol. Chem., 272, 52019–52024

72. Perez, J. L., Khatli, L., Chang, C., Srivastava, S., Osten, P., and Ziff, E. B. (2001) PICK1 targets activated protein kinase C alpha to AMPA receptor clusters in spines of hippocampal neurons and reduces surface levels of the AMPA-type glutamate receptor subunit 2. J. Neurosci., 21, 5417–5428

73. Dev, K. K., Nakajima, Y., Kitano, J., Braithwaite, S. P., Lenne, P. F., Olive, D., and Newton, A. C. (2011) Protein kinase C alpha promotes cell migration through a PDZ-dependent interaction with its novel substrate discs large homolog 1 (DLG1). J. Biol. Chem., 286, 43559–43568

74. Ron, D., Chen, C. H., Caldwell, J., Jamieson, L., Orr, E., and Mochly-Rosen, D. (1994) Cloning of an intracellular receptor for protein kinase C. Isoenzyme-dependent regulation of kinase activity by the caveolin scaffolding domain peptide. J. Biol. Chem., 269, 11786–11787

75. Ron, D., Chen, C. H., Caldwell, J., Jamieson, L., Orr, E., and Mochly-Rosen, D. (1994) Cloning of an intracellular receptor for protein kinase C: a homolog of the beta subunit of G proteins. Proc. Natl. Acad. Sci. U.S.A., 91, 839–843

76. Csukai, M., Chen, C. H., De Matteis, M. A., and Moehly-Rosen, D. (1997) The coatamer protein beta-COP, a selective binding protein (RACK) for protein kinase C epsilon. J. Biol. Chem., 272, 29200–29206

77. Hirsch, A. H., Glantz, S. B., Li, Y., You, Y., and Rubin, C. S. (1992) Cloning and expression of an intron-less gene for AKAP75, an anchor protein for the regulatory subunit of cAMP-dependent protein kinase II beta. J. Biol. Chem., 267, 2131–2134

78. Hirschi, K. K., Smith, F. D., McNamara, C., and Langeberg, L. K., and Scott, J. D. (2004) AKAP-Lbc nucleates a protein kinase D activation scaffold. Mol. Cell, 15, 889–899

79. Klauk, T. M., Faux, M. C., Labudda, K., Langeberg, L. K., Jaken, S., and Scott, J. D. (1996) Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. Science, 271, 1589–1592

80. Hoshi, N., Langeberg, L. K., Gould, C. M., Newton, A. C., and Scott, J. D. (2010) Interaction with AKAP79 modifies the cellular pharmacology of PKC. Mol. Cell, 37, 541–550

81. Klauck, T. M., Faux, M. C., Labudda, K., Langeberg, L. K., Jaken, S., and Scott, J. D. (1996) Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. Science, 271, 1589–1592

82. Hoshi, N., Langeberg, L. K., Gould, C. M., Newton, A. C., and Scott, J. D. (2010) Interaction with AKAP79 modifies the cellular pharmacology of PKC. Mol. Cell, 37, 541–550

83. Leitges, M., Kovic, J., Plomann, M., and Linden, D. J. (2004) A unique PDZ ligand in PKC alpha confers induction of cerebellar long-term synaptic depression. Neuron, 44, 585–594

84. Steudinger, J., Zhuo, Z., Burgess, R., Elledge, S. J., and Olson, E. N. (1995) PICK1: a perinuclear binding protein and substrate for protein kinase C isolated by the yeast two-hybrid system. J. Cell Biol., 128, 263–271

85. Steudinger, J., Lu, J., and Olson, E. N. (1997) Specific interaction of the PDZ domain protein PICK1 with the COOH terminus of protein kinase C-alpha. J. Biol. Chem., 272, 52019–52024
mation of chicken cells by the gene encoding the catalytic subunit of PI 3-kinase. Science 276, 1848–1850
94. Regala, R. P., Heems, C., Jamiesson, L., Khoo, A., Edell, E. S., Lohse, C. M., and Fields, A. P. (2005) Atypical protein kinase C iota is an oncogene in human non-small cell lung cancer. Cancer Res. 65, 8905–8911
95. Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S. I., Puc, J., Miller, C., Rodgers, L., McCombie, R., Bignon, S. H., Giovanello, B. C., Ittmann, M., Tycko, B., Hibshoosh, H., Wigler, M. H., and Parsons, R. (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275, 1943–1947
96. Steck, P. A., Pershouse, M. A., Jasser, S. A., Yung, W. K., Lin, H., Ligon, A. H., Langford, L. A., Baumgard, M. L., Hattier, T., Davis, T., Frye, C., Hu, R., Sweidlund, B., Teng, D. H., and Tavtigian, S. V. (1997) Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet. 15, 356–362
97. Li, D. M., and Sun, H. (1997) TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. Cancer Res. 57, 2124–2129
98. O’Costa, A. M., Robinson, J. K., Maududi, T., Chaturvedi, V., Nickoloff, B. J., and Denning, M. F. (2006) The proapoptotic tumor suppressor protein kinase C-delta is lost in human squamous cell carcinomas. Oncogene 25, 378–386
99. Berrar, D., Sturgeon, B., Bradbury, I., Downes, C. S., and Dubitzky, W. (2005) Survival trees for analyzing clinical outcome in lung adenocarcinomas based on gene expression profiles: identification of neogenin and diacylglycerol kinase alpha expression as critical factors. J. Comput. Biol. 12, 534–544
100. Guo, R., Wan, C. K., Carpenter, J. H., Mousallem, T., Boustany, R. M., Kuan, C. T., Burks, A. W., and Zhong, X. P. (2008) Synergistic control of T cell development and tumor suppression by diacylglycerol kinase alpha and zeta. Proc. Natl. Acad. Sci. U.S.A. 105, 11909–11914
101. Vogt, P. K., Kang, S., Eislinger, M. A., and Gynmopoulos, M. (2007) Cancer-specific mutations in phosphatidylinositol 3-kinase. Trends Biochem. Sci. 32, 342–349
102. Chow, L. M., and Baker, S. J. (2006) PTEN function in normal and neoplastic growth. Cancer Lett. 241, 184–196
103. Ali, I. U., Schriml, L. M., and Dean, M. (1999) Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. J. Natl. Cancer Inst. 91, 1922–1932
104. Stemke-Hale, K., Gonzalez-Angulo, A. M., Lu, A., Neve, R. M., Kuo, W. L., Davies, M., Carey, M., Hu, Z., Guan, Y., Sahin, A., Symmans, W. F., Pusztai, L., Nolden, L. K., Horling, H., Berns, K., Hung, M. C., van de Vijver, M. J., Valero, V., Gray, J. W., Bernards, R., Mills, G. B., and Hennessey, B. T. (2008) An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. Cancer Res. 68, 6084–6091
105. Yamada, T., Takeuchi, S., Fujita, N., Nakamura, A., Wang, W., Li, Q., Oda, M., Mitsudomi, T., Yatabe, Y., Sekido, Y., Yoshida, J., Higashiyama, M., Noguchi, M., Uehara, H., Nishioka, Y., Sone, S., and Yano, S. (2013) Akt kinase-interacting protein1, a novel therapeutic target for lung cancer with EGFR-activating and gatekeeper mutations. Oncogene 32, 4427–4435
106. Shayeesteh, L., Lu, Y., Kuo, W. L., Baldocchi, R., Godfrey, T., Collins, C., Pinkel, D., Powell, B., Mills, G. B., and Gray, J. W. (1999) PIK3CA is implicated as an oncogene in ovarian cancer. Nat. Genet. 21, 99–102
107. Ma, Y. Y., Wei, S. J., Lin, Y. C., Lung, J. C., Chang, T. C., Wang-Peng, J., Liu, J. M., Yang, D. M., Yang, W. K., and Shen, C. Y. (2008) PIK3CA as an oncogene in cervical cancer. Oncogene 27, 2739–2744
108. Bellacosa, A., de Feo, D., Godwin, A. K., Bell, D. W., Cheng, J. Q., Altvor, D. A., Wan, M., Dubeau, L., Scambia, G., Masciullo, V., Ferrandina, G., Benedetti Panici, P., Mancuso, S., Neri, G., and Testa, J. R. (1995) Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. Int. J. Cancer 64, 280–285
109. Cheng, J. Q., Ruggeri, B., Klein, W. M., Sonoda, G., Altvor, D. A., Watson, D. K., and Testa, J. R. (1999) Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. Proc. Natl. Acad. Sci. U.S.A. 96, 3636–3641
110. Taylor, B. S., Schultz, N., Hieronymus, H., Gopalan, A., Xiao, Y., Carver, B. S., Arora, V. K., Kaushik, P., Cerami, E., Reva, B., Antipin, Y., Mitsiades, N., Landers, T., Dolgalev, I., Major, J. E., Wilson, M., Socci, N. D., Lash, A. E., Heguy, A., Eastham, J. A., Scher, H. I., Reuter, V. E., Scardino, P. T., Sander, C., Sawyer, C. L., and Gerald, W. L. (2010) Integrative genomic profiling of human prostate cancer. Cancer Cell 18, 11–22
111. Gallegos, L. L., and Newton, A. C. (2008) Spatiotemporal dynamics of lipid signaling: protein kinase C as a paradigm. IUBMB Life 60, 782–789