Vitamin E Facilitates the Inactivation of the Kinase Akt by the Phosphatase PHLPP1

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Vitamin E is a fat-soluble vitamin with antioxidant properties. Tocopherols are the predominant form of vitamin E found in the diet and in supplements and have garnered interest for their potential cancer therapeutic and preventive effects, such as the dephosphorylation of Akt, a serine/threonine kinase with a pivotal role in cell growth, survival, and metabolism. Dephosphorylation of Akt at Ser473 substantially reduces its catalytic activity and inhibits downstream signaling. We found that the mechanism by which α-tocopherol and γ-tocopherol facilitate this site-specific dephosphorylation of Akt was mediated through the pleckstrin homology (PH) domain–dependent recruitment of Akt and PHLPP1 (PH domain leucine-rich repeat protein phosphatase 1, isoform 1) to the plasma membrane. We structurally optimized these tocopherols to generate a series of potent Akt pathway targeted agents.

INTRODUCTION

Although the Selenium and Vitamin E Cancer Prevention Trial (SELECT) failed to demonstrate the chemopreventive effect of α-tocopherol in prostate cancer (1), considerable interest still exists in evaluating the antitumorigenic effects of γ-tocopherol and other forms of tocopherol in light of their superior anti-inflammatory and antitumor efficacies (2). For example, γ-tocopherol exhibits greater potency than α-tocopherol in suppressing prostate cancer cell proliferation (3) and carcinogen-induced transformation of murine fibroblasts (4). From a translational perspective, a major impediment to the clinical development of vitamin E for cancer prevention is a lack of understanding of the molecular target by which tocopherols mediate antiproliferative effects. Evidence has implicated various mechanisms by which tocopherols perturb cancer cell function and survival independent of antioxidant properties (2, 5). Among these, dephosphorylation of Akt by tocopherols, though at high concentrations, is especially noteworthy in light of the role of Akt signaling in mediating cancer cell survival (6, 7).

Here, we report that α-tocopherol and γ-tocopherol can mediate the site-specific dephosphorylation of the kinase Akt at Ser473 with activities paralleling their respective antiproliferative potencies in prostate cancer cells. Moreover, this selective Akt dephosphorylation is attributable to a mechanism whereby α-tocopherol and γ-tocopherol facilitate the co-recruitment of Akt and pleckstrin homology (PH) domain leucine-rich repeat protein phosphatase 1 (PHLPP1), a Ser473-specific Akt protein phosphatase, to the plasma membrane through PH domain recognition. This tocopherol-induced activation of PHLPP1 is noteworthy in light of the tumor suppressor role of PHLPP1 in prostate cancer by counteracting the functional loss of phosphatase and tensin homolog (PTEN) in suppressing Akt activation (8). Moreover, structural modification of these tocopherols enhanced this activity, thereby providing a rationale for optimizing tocopherols to generate a series of potent Akt pathway–targeted agents.

RESULTS

α-Tocopherol and γ-tocopherol mediate the site-specific dephosphorylation of Akt at Ser473

We examined the antiproliferative activities of α-tocopherol and γ-tocopherol in two prostate cancer cell lines, LNCaP (androgen-responsive) and PC-3 (androgen-independent), both of which exhibit activated Akt resulting from loss of PTEN function. Both cell lines were equally susceptible to the antiproliferative effect of these compounds, and γ-tocopherol [median inhibitory concentration (IC50), ~100 to 150 μM] was more potent than α-tocopherol (IC50 ~400 μM) (Fig. 1A). This cytotoxic effect was attributable to apoptosis (Fig. 1B) and was cancer cell–specific because normal prostate epithelial cells were resistant to apoptosis induced by either α-tocopherol or γ-tocopherol (fig. S1A). Because vitamin E inhibits cancer cell proliferation by targeting Akt (6, 7), we examined the effects of α-tocopherol and γ-tocopherol on the phosphorylation of Akt in both cell lines. Both tocopherols dose-dependently reduced the phosphorylation...
Tocopherols promote the dephosphorylation of Ser<sup>473</sup> in Akt by recruiting Akt and PHLPP1 to the plasma membrane

These findings suggest that the antitumor action for the tocopherols occurs through the site-specific dephosphorylation and consequent inactivation of Akt. Because tocopherols are localized within the membrane after uptake by cells, we postulated that this tocopherol-facilitated dephosphorylation of Ser<sup>473</sup> in Akt would be a membrane-associated event. In support of this premise, immunocytochemical analysis showed that Akt localized to the membrane in response to α-tocopherol and γ-tocopherol in LNCaP cells (Fig. 1, D and E, and fig. S1D). This tocopherol-mediated membrane localization of Akt is similar to the reported phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)<sub>6</sub>)-facilitated recruitment of Akt through PH domain recognition (9, 10). Thus, radiometric analysis of phosphoinositide production in tocopherol-treated LNCaP cells was performed, which showed that PI(3,4,5)<sub>6</sub> concentrations were unaffected by exposure to 500 μM α-tocopherol (fig. S2A). In addition, pretreatment of LNCaP cells with the phosphoinositide 3-kinase (PI3K) inhibitor LY-294002 did not affect the ability of α-tocopherol and γ-tocopherol to facilitate the membrane recruitment of Akt (fig. S2B). Together, these findings suggest that PI(3,4,5)<sub>6</sub> is not required for tocopherol-induced Akt recruitment. Moreover, because the rictor-mTOR (mammalian target of rapamycin) complex (mTORC2) facilitates phosphorylation of Akt at Ser<sup>473</sup> in many types of cells (11, 12), we examined the concentration-dependent effects of α-tocopherol and γ-tocopherol on the abundance of mTOR and rictor and the phosphorylation of the downstream targets of mTORC2, including serum- and glucocorticoid-inducible kinase (SGK) and protein kinase Cα (PKCα). The data show that α-tocopherol and γ-tocopherol had no effect on the phosphorylation of SGK or PKCα (fig. S2C), suggesting that mTORC2 is not involved in tocopherol-induced dephosphorylation of Akt.

Compared to Akt activation, the mechanisms by which Akt is inactivated are less well defined (13). Protein phosphatase 2A and PHLPP1 facilitate the dephosphorylation of Akt at Thr<sup>308</sup> and Ser<sup>473</sup> (14–16), respectively. Because PHLPP1, like Akt, also contains a PH domain, we hypothesized that α-tocopherol and γ-tocopherol induced the recruitment of both Akt and PHLPP1 to the plasma membrane through their respective PH domains, leading to their colocalization and subsequent dephosphorylation of Ser<sup>473</sup> in Akt. Immunocytochemical analysis showed that α-tocopherol and γ-tocopherol facilitated the membrane localization of PHLPP1 (Fig. 2, A and B, and fig. S2D) in a manner similar to that of Akt in LNCaP cells. Western blot analysis of the membrane fraction of treated cells confirmed that α-tocopherol and γ-tocopherol increased the membrane localization of Akt and PHLPP1 in a dose-dependent manner, which was accompanied by parallel decreases in phosphorylation of Ser<sup>473</sup> in Akt and the abundance of cytoplasmic Akt and PHLPP1 (Fig. 2C and fig. S2E). Subsequent communoprecipitation analysis of Akt-PHLPP1 complexes from the membrane fraction revealed dose-dependent increases in the association of these two proteins in response to α-tocopherol and γ-tocopherol (Fig. 2D and fig. S2E).
From a mechanistic perspective, these findings contrast with the general notion that Akt membrane translocation is integral to its activation by phosphorylation at Thr\(^{308}\) and Ser\(^{473}\) by phosphoinositide-dependent protein kinase 1 (PDK1) and mTORC2, respectively.

**Truncation of the aliphatic side chain enhances the ability of α-tocopherol and γ-tocopherol to induce apoptotic death through inactivation of Akt and membrane recruitment of Akt and PHLPP1**

On the basis of the above findings, we proposed that the ability of α-tocopherol and γ-tocopherol to recruit Akt and PHLPP1 to the plasma membrane was mediated through interactions of the chroman ring in the polar head group with these proteins at the membrane-cytosol interface. Specifically, we proposed a thermodynamic interplay between the long aliphatic side chain of α-tocopherol or γ-tocopherol and the lipid bilayer membrane, which might restrict the accessibility of the chroman ring to the cytosolic milieu by pulling the head group inward into the membrane. Thus, we hypothesized that shortening the side chain of the tocopherols would increase the cytoplasmic exposure of the respective polar head groups, thereby enhancing their ability to recruit Akt and PHLPP1 and thus increasing the tocopherols’ antitumor activities.

The proof of concept of this hypothesis was provided by α-VE5 and γ-VE5, which were derived from α-tocopherol and γ-tocopherol, respectively, by removing two isopropyl units from the respective aliphatic side chains (Fig. 3A). α-VE5 and γ-VE5 were an order of magnitude more potent than their parent molecules in suppressing the viability of LNCaP and PC-3 cells (IC\(_{50}\) of α-VE5, 15 to 20 μM; IC\(_{50}\) of γ-VE5, 7 to 10 μM) (Fig. 3A) through apoptosis (Fig. 3B), accompanied by parallel reduction in the phosphorylation of Akt at Ser\(^{473}\) without disturbing that of Thr\(^{308}\) (Fig. 3C and Fig. S3, A and B). The involvement of this Akt inactivation in α-VE5– and γ-VE5–induced cell death was validated by the partially protective effect of ectopic expression of CA-Akt (Akt T308D and S473D; T308D/S473D) (Fig. 3D). As with α-tocopherol and γ-tocopherol, α-VE5– and γ-VE5–mediated inhibition of phosphorylation of Ser\(^{473}\) in Akt was not associated with inhibition of mTORC2 as shown by the lack of changes in the abundance or phosphorylation of mTOR, raptor, SGK, and PDKCo in drug-treated cells (fig. S2C). Moreover, normal prostate epithelial cells were more resistant to α-VE5 and γ-VE5 than malignant cells with IC\(_{50}\) values of about 50 and 40 μM, respectively (fig. S1A), which represent a three- to fivefold difference in potency relative to LNCaP and PC-3 cells.

Furthermore, immunocytochemical analysis revealed that α-VE5 and γ-VE5 retained the ability of the tocopherols (Figs. 1D and 2A) to facilitate the membrane recruitment of Akt and PHLPP1 (Fig. 4, A to C, and Fig. S4, A and B). This immunocytochemical finding was confirmed by Western blot analysis, which showed that exposure of LNCaP cells to α-VE5 or γ-VE5 led to concentration-dependent increases in membrane-associated Akt and PHLPP1 and parallel decreases in their cytoplasmic abundance as well as a decrease in phosphorylation of Ser\(^{473}\) in Akt (Fig. 4D and Fig. S4C). In addition, co-immunoprecipitation analysis demonstrated concentration-dependent increases in Akt-PHLPP1 complex formation in α-VE5– and γ-VE5–treated cells, further substantiating the role of PHLPP1 in facilitating drug-induced dephosphorylation of Ser\(^{473}\) in Akt (Fig. 4E and Fig. S4C).

PIP\(_{2}\)-induced membrane recruitment and activation of Akt is mediated through cholesterol-rich lipid rafts of the plasma membrane (17–20). Data on the distribution of α-tocopherol within the cell membrane suggest a nonrandom distribution (21, 22), with some studies indicating its association with lipid rafts (22), and others suggesting that it is localized to polyunsaturated fatty acid-rich nonraft domains (21). Consequently, we investigated whether Akt and PHLPP1 localized to the lipid raft or nonraft microdomain of cell membranes in response to α-VE5 and γ-VE5 using density gradient...
PI(3)-mediated recruitment of Akt to membrane raft domains.

that this VE5-induced reduction in cholesterol content might hamper the raft domains (fractions 3 and 4) by as much as 30% (Fig. 5B), suggesting nonraft fractions and was accompanied by a parallel decrease by more than 2.5-fold in these treatment groups decreased cell death and the dephosphorylation of Ser473 in Akt in LNCaP cells. Silencing of PHLPP1 expression partially protected cells against the inhibitory effects of γ-VE5 on cell viability and phosphorylation of Akt at Ser473 (Fig. 5C and fig. S5B). Similar findings were obtained for α-VE5-treated cells (fig. SSC).

γ-VE5 suppresses PC-3 and LNCaP-abi xenograft tumor growth in vivo

The effects of γ-VE5 on tumor growth in vivo were assessed in athymic nude mice bearing subcutaneous xenograft tumors generated from luciferase-expressing PC-3 (PC-3-luc) and LNCaP-abi (an androgen-independent LNCaP subline) cells. LNCaP-abi cells and the parental LNCaP cells were comparably susceptible to the antiproliferative effects of α-VE5 and γ-VE5 (IC50, 10 and 7 μM, respectively; fig. S5D). Mice bearing tumors established from PC-3-luc cells or LNCaP-abi cells were injected with γ-VE5 or vehicle. Measurements of bioluminescence revealed that treatment with γ-VE5 inhibited PC-3-luc tumor growth relative to vehicle-treated controls at 21 days (Fig. 5D). Additionally, γ-VE5 suppressed phosphorylation of Akt at Ser473 without disrupting that at Thr308 (Fig. 5D) in tumors. Furthermore, the phosphorylation of two Akt downstream targets, murine double minute 2 (MDM2) and inhibitor of nuclear factor κB kinase

centrifugation. As indicated by the presence of the raft-associated marker flotillin-2, lipid rafts were associated with the low-density fractions, whereas, reminiscent of another report (23), most of Akt and all detectable Akt phosphorylated at Ser473 were present in higher-density fractions that corresponded to the nonraft membrane in vehicle-treated cells (Fig. 5A and fig. S5A). Moreover, only a small amount of PHLPP1 was found in these Akt-containing, nonraft membrane fractions in vehicle-treated cells. However, after exposure to α-VE5 and γ-VE5, the association of PHLPP1 with the membrane increased more than 2.5-fold in these nonraft fractions and was accompanied by a parallel decrease by more than 65% in the phosphorylation of Ser473 in Akt (Fig. 5A). This finding is consistent with the hypothesis that α-tocopherol is localized to polyunsaturated fatty acid-rich nonraft domains (27).

In light of our finding that α-VE5 and γ-VE5 facilitated Akt recruitment preferentially to the nonraft domains without observable increases in Akt binding to raft domains and the evidence suggesting that cholesterol in raft microdomains plays a critical role in facilitating Akt membrane recruitment and activation (18, 19, 23, 24), we examined the effect of α-VE5 and γ-VE5 on the cholesterol content of individual membrane fractions. Both α-VE5 and γ-VE5 reduced the cholesterol content in the raft domains (fractions 3 and 4) by as much as 30% (Fig. 5B), suggesting that this VE5-induced reduction in cholesterol content might hamper the PI3-γ-mediated recruitment of Akt to membrane raft domains.

To further corroborate the role of PHLPP1 in mediating tocopherol-induced Akt inactivation, we examined the effect of small interfering RNA (siRNA)-mediated knockdown of PHLPP1 on α-VE5- and γ-VE5-induced subunit (lKKK), was decreased, thus confirming that γ-VE5 inhibited Akt signaling in tumors (Fig. 5D). Similar findings were obtained in LNCaP-abi tumor-bearing mice in which daily treatment with γ-VE5 significantly inhibited tumor growth relative to vehicle-treated controls (fig. S5E). As with the PC-3-luc tumors, this suppressive effect on LNCaP-abi tumor growth was also associated with the inhibition of Akt signaling, evidenced by the reduced phosphorylation of Akt at Ser473, MDM2, and IKKα in tumors from γ-VE5–treated mice relative to vehicle-treated controls (fig. S5E).

The toxicologic effects of γ-VE5 in PC-3-luc tumor-bearing mice were assessed by body weight, pathologic, and hematologic evaluations. Mean body weights in both vehicle- and γ-VE5–treated groups decreased slightly but insignificantly over the treatment period (table S1). Gross pathology findings at necropsy were limited to the presence of abdominal adhesions and variable amounts of clear fluid within the abdomen in γ-VE5–treated mice. Hematological findings after treatment included reductions in hematocrit, red blood cell number, and hemoglobin concentration in γ-VE5–treated mice relative to controls (table S1). Serum chemistry analysis revealed an increase in aspartate aminotransferase and a decrease in serum albumin concentrations in γ-VE5–treated mice (table S2). Nonetheless, the values of the affected parameters were within the normal ranges for mice; thus, the clinical relevance of these changes is unclear.

PH domain recognition by tocopherol and VE5 is selective for Akt and PHLPP1

We hypothesized that the tocopherols and corresponding VE5 derivatives mediated the membrane translocation of Akt and PHLPP1 through the
The impact of these agents on the intracellular distribution of other PH domain–containing kinases, including PDK1, integrin-linked kinase (ILK) (25), and Bruton’s tyrosine kinase (BTK), by immunocytochemistry. No changes were apparent in the distribution of these kinases in response to either tocopherol (Fig. 6C and fig. S6C), indicating a high degree of selectivity in PH domain recognition by the tocopherols and VE5 compounds at the indicated concentrations. To determine whether the membrane recruitment of these proteins was a concentration-dependent phenomenon, we assessed the time-dependent membrane recruitment of PDK1 at higher γ-VE5 concentrations. At 25 and 35 μM γ-VE5, membrane-associated PDK1 accounted for about 50% and greater than 90%, respectively, of the total immunofluorescence signal for PDK1 after 6 hours of exposure (longer exposure led to cell detachment) (fig. S7), indicating a concentration-dependent control of membrane recruitment.

Next, we used surface plasmon resonance (SPR) spectroscopy to measure the binding affinities of α-VE5 and γ-VE5 for the glutathione S-transferase (GST)–tagged PH domains of Akt and PHLP1 compared with those of PDK1 and ILK. Unfortunately, the poor solubility of the tocopherols prohibited their use in SPR analysis because of the formation of oil droplets. For each of the PH domains, the dissociation constants (Ka) for α-VE5 and γ-VE5 were determined (table S3). These data reveal that the binding affinities of α-VE5 and γ-VE5 for the PH domains of Akt and PHLP1 paralleled their relative potencies in facilitating dephosphorylation of Ser473 in Akt and apoptosis in LNCaP cells and were 6- to 29-fold greater than those for the PH domains of PDK1 and ILK. This finding underscores the selectivity of α-VE5 and γ-VE5 in facilitating membrane recruitment among PH domain–containing proteins.

To compare the recognition profiles of PIP3 with those of α-VE5 or γ-VE5 for the PH domains of Akt and other PH domain–containing proteins, we used dioctanoyl PIP3, a soluble form of PIP3, as ligand for SPR analysis. Of the PH domains tested, PIP3 exhibited the highest selectivity for PH domain–containing Akt and PHLPP1 with equal potency, PIP3 exhibited differential binding to these domains of Akt and PHLPP1 compared to PH domains of PDK1 and ILK. Unfortunately, the poor solubility of the tocopherols prohibited their use in SPR analysis because of the formation of oil droplets. For each of the PH domains, the dissociation constants (Ka) for α-VE5 and γ-VE5 were determined (table S3). These data reveal that the binding affinities of α-VE5 and γ-VE5 for the PH domains of Akt and PHLP1 paralleled their relative potencies in facilitating dephosphorylation of Ser473 in Akt and apoptosis in LNCaP cells and were 6- to 29-fold greater than those for the PH domains of PDK1 and ILK. This finding underscores the selectivity of α-VE5 and γ-VE5 in facilitating membrane recruitment among PH domain–containing proteins.

Interactions within the PH domain VL2 loop underlie the structural basis for the differential ligand recognition of α-VE5 compared to γ-VE5 by the Akt-PH domain

To envisage the mode of ligand recognition between α-VE5 or γ-VE5 and the PH domain of Akt, we carried out a modeling analysis using the
reported x-ray structure of the PH domain of Akt (27, 28) (fig. S8A). Docking of α-VE5 and γ-VE5 into the PH domain suggests that γ-VE5 favored the binding pocket formed within the VL2 loop, in which hydrogen bonding between the OH group of the chroman ring and the peptide backbone of Ala50-Pro51 played a crucial role (Fig. 7, A and B). This docking analysis also suggests that the lower binding affinity of α-VE5 (Kd, 3.4 μM) relative to γ-VE5 (Kd, 0.9 μM) for the Akt-PH domain might be attributable to steric factors. Unlike γ-VE5, α-VE5 has a methyl group at position 5 of the chroman ring, which might impose steric repulsion with the phenyl ring of the Tyr38 residue (Fig. 7B). To corroborate this hypothesis, we replaced Tyr38 with glycine using site-directed mutagenesis, generating the Y38G PH domain mutant. SPR analysis revealed that this mutation significantly enhanced the binding affinities of α-VE5 and, to a lesser extent, γ-VE5 for the PH domain (table S3). Together, these findings support the proposed mode of ligand recognition of α-VE5 and γ-VE5 by the Akt-PH domain (Fig. 7B).
These findings, however, raise the question of how this differential recruitment to distinct membrane regions occurs in light of the three orders of magnitude difference between the binding affinity of PIP3 and that of α-tocopherol and γ-tocopherol and α-VE5 and γ-VE5 for Akt (nanomolar compared with micromolar). In this regard, membrane cholesterol content may play an important role. The PIP3-mediated membrane recruitment and activation of Akt not only involves the complex protein-lipid interaction between Akt and PIP3 but also requires the coordinated action of cholesterol and sphingolipids to facilitate the formation of raft microdomains (18, 19, 23, 24). Moreover, reducing the cellular cholesterol content inhibits activation of Akt in lipid rafts (18, 23). Thus, it is plausible that the suppressive effect of α-VE5 and γ-VE5 on the cholesterol content of lipid raft membrane fractions (Fig. 5B) reduced PIP3-mediated membrane recruitment of Akt to raft microdomains.

In addition, we speculate that PIP3 might exhibit differential binding affinities for the dephosphorylated and phosphorylated forms of Akt. Despite elevated PIP3 abundance in PTEN-negative LNCaP cells, most of Akt resided in the cytoplasm (Figs. 1D and 4A), which is in line with the general notion that Akt, once phosphorylated, is released from the membrane to interact with and phosphorylate target proteins in the cytoplasm and nucleus. Nevertheless, this cytoplasmic pool of phosphorylated Akt was responsive to tocopherol- or VE5-induced membrane localization to nonraft domains and consequent dephosphorylation. This possible difference in the mode of recognition of Akt by tocopherols or VE5 compared to PIP3, along with the aforementioned reductions in cholesterol content of raft microdomains, may underlie the net dephosphorylation of Akt in tocopherol- or VE5-treated cells.

In light of the tumor suppressor role of PHLPP1 in blocking PTEN-mutant prostate cancer progression (8) and in mediating androgen receptor–induced inhibition of Akt (29, 30), PHLPP1 activation represents a therapeutically relevant target for prostate cancer. However, from a chemopreventive perspective, the high concentrations required for α-tocopherol and γ-tocopherol to induce PHLPP1-mediated Akt inhibition (greater than 150 μM) might not be attainable in humans through vitamin E supplementation. The human diet provides mainly α-tocopherol and γ-tocopherol, whereas supplements generally supply vitamin E as α-tocopherol acetate in racemic form (all-rac-α-tocopherol acetate). Because of its hydrophobic nature, the intestinal absorption, plasma transport, and cellular uptake of vitamin E require protein-mediated processes that involve transporters, such as the scavenger receptor class B type I, and various plasma lipoproteins (22), which might represent a limiting factor for achieving high plasma concentrations of vitamin E. For example, vitamin E concentration in the plasma reaches a plateau at 600 mg of daily supplementation (31). In light of the 10-fold higher potency, α-VE5 and γ-VE5 provide a proof of concept that PHLPP1 is a “druggable” target, which is supported by the in vivo efficacy of γ-VE5 in suppressing the growth of PC-3 and LNCaP-abl xenograft tumors in athymic nude mice (Fig. 5D and fig. S5E).

We hypothesize that the hydrophobicity of the long aliphatic side chains of the tocopherols limits their access to the PH domains of Akt and PHLPP1 at the membrane-cytoplasm interface. Thus, truncation of the side chains by two isoprenyl units endowed α-VE5 and γ-VE5 with a more favorable physicochemical property compared to α-tocopherol and γ-tocopherol for the membrane recruitment of the target proteins, resulting in greater

**DISCUSSION**

Here, we obtained evidence that α-tocopherol and γ-tocopherol mediate the dephosphorylation of Ser473 in Akt in PTEN-negative LNCaP and PC-3 cells through membrane colocalization with PHLPP1. Although tocopherols and PIP3 share the ability to recruit Akt to the plasma membrane, they lead to opposite effects on Akt functional status because of differences in PH domain recognition. Although it is not thought to be confined exclusively to specific membrane microdomains, PIP3 can be incorporated into raft microdomains formed in coordination with cholesterol and sphingolipids upon Akt-PH domain binding (19), where it recruits PDK1 through its PH domain to catalyze phosphorylation of Akt at Thr308 (20). In contrast, our findings show that tocopherols selectively interact with PHLPP1 to cause dephosphorylation of Akt at Ser473 in nonraft microdomains (Fig. 7C). Indeed, our results suggest that PIP3- and tocopherol-facilitated Akt recruitment occurs in distinct regions (raft or nonraft microdomains) of the cytoplasmic membrane of LNCaP cells, which is consistent with a report that most of membrane-associated Akt is present in the phosphorylated form in the nonraft domains of LNCaP cells (23).

These findings, however, raise the question of how this differential recruitment to distinct membrane regions occurs in light of the three orders of magnitude difference between the binding affinity of PIP3 and that of α-tocopherol and γ-tocopherol and α-VE5 and γ-VE5 for Akt (nanomolar compared with micromolar). In this regard, membrane cholesterol content may play an important role. The PIP3-mediated membrane recruitment and activation of Akt not only involves the complex protein-lipid interaction between Akt and PIP3 but also requires the coordinated action of cholesterol and sphingolipids to facilitate the formation of raft microdomains (18, 19, 23, 24). Moreover, reducing the cellular cholesterol content inhibits activation of Akt in lipid rafts (18, 23). Thus, it is plausible that the suppressive effect of α-VE5 and γ-VE5 on the cholesterol content of lipid raft membrane fractions (Fig. 5B) reduced PIP3-mediated membrane recruitment of Akt to raft microdomains.

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dephosphorylation of Akt. The membrane targeting of PHLPP1 and its dephosphorylation of Akt depend on the protein Scrib, which facilitates the formation of a PHLPP1-Akt-Scrib heterotrimeric complex at the cell membrane (32). Because Scrib deficiency contributes to prostate tumorigenesis in preclinical models and its deregulation is associated with poor prognosis in human prostate cancer patients (33), the use of PH domain–containing agents, such as α-VE5 and γ-VE5, may yield therapeutic benefits by restoring PHLPP1-mediated dephosphorylation of Akt in prostate tumors with dysfunctional Scrib.

This study focused on PHLPP1; however, the PHLPP family consists of two isoforms, PHLPP1 and PHLPP2, which dephosphorylate distinct isoforms of Akt (15). Despite these different but overlapping specificities for Akt isoforms, PHLPP1 and PHLPP2 exhibit nearly identical domain structures, greater than 60% amino acid sequence homology in the PH domain, and similarities in cellular localization (15). Thus, we expect that PHLPP2, like PHLPP1, can also bind to the polar head group of the tocopherols and VE5 compounds, leading to membrane recruitment and consequent Akt dephosphorylation. Nevertheless, differences between the two isoforms have been demonstrated not only with respect to Akt isoform specificity but also in the regulation of their phosphatase activities and in the possible prognostic value of PHLPP1, but not PHLPP2, in pancreatic cancer patients (34). A complete understanding of the involvement of PHLPP2 in tocopherol’s effects on Akt signaling requires investigation, which is ongoing in our laboratory.

Consistent with its predicted activity against tumors with activated Akt status, γ-VE5 as a single agent displayed strong tumor-suppressive activity domain–containing proteins in response to α-VE5 and γ-VE5 treatment (Figs. 4 and 6C). We rationalize that, because α-VE5 and γ-VE5 passively diffuse into the plasma membrane, the local concentration at the membrane-cytosol interface becomes a limiting factor that controls the recruitment of PH domain–containing proteins from the cytoplasm. For instance, γ-VE5 at 15 μM presumably achieves local concentrations at the membrane that are high enough to facilitate the localization of Akt and PHLPP1 (Fig. 4) but are insufficient for the recruitment of the low-affinity PDK1 and ILK (Fig. 6C). This concentration-dependent control of membrane recruitment was corroborated by the time-dependent increase in membrane localization of PDK1 in response to higher concentrations of γ-VE5 (fig. S7).

The preferential binding of α-VE5 and γ-VE5 to the PH domain of Akt relative to those of PDK1, ILK, and BTK is intriguing. Each PH domain contains a sequence homologous to that of the VL2 loop of the PH domain of Akt (AVYKERPOQDVRSEQAPL38); PDK1, 106-VDPVPNKLGEIPWSQ120; ILK, 198-KLNENHSELWKG112; BTK, 379-LSYYEYDFERGGRCS151 (fig. SSB). However, conformational analysis revealed differences in the secondary structures between the Akt VL2 loop and these other sequences. Specifically, the PH domains of PDK1, BTK, and ILK contain β sheet structures in lieu of a variable loop structure (fig. SSB). Thus, this difference in their secondary structures might underlie the ability of tocopherol and the VE5 derivatives to discriminate between Akt and these other PH domain–containing proteins.

In summary, this study describes a mechanism by which vitamin E mediates dephosphorylation of Ser473 in Akt in cancer cells. This mode of Akt inhibition is different from that of kinase inhibitors or PH domain–

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**Fig. 7.** Molecular modeling of the binding of α-VE5 and γ-VE5 with the Akt-PH domain. (A) A model for the docking of γ-VE5 into the VL2 loop of the PH domain of Akt. Binding of PIP3 is also represented. (B) Modeled interactions of γ- and α-VE5 with amino acid residues of the VL2 loop of the Akt-PH domain. VE5 compounds (green) and hydrogen bonding (red dashed line) are indicated. (C) Diagram depicting the mechanism of γ-tocopherol-mediated dephosphorylation of Ser473 in Akt compared to that of PIP3-mediated Akt activation in cancer cells.

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in vivo against the growth of subcutaneous xenograft tumors established from the PTEN-null PC-3 and LNCaP-abl prostate cancer cell lines. The abdominal adhesions and ascites in γ-VE5–treated mice were possibly a response to chronic irritation and peritonitis associated with repeated daily intraperitoneal injections of the compound, and may underlie the mild weight loss observed in these mice. Although complete blood counts revealed indications of anemia, the clinical relevance of these findings is unclear because these values were all within normal limits for mice. Similarly, the affected concentrations of aspartate aminotransferase and albumin were also within normal limits and were not associated with changes in other indicators of liver function. Studies designed to more thoroughly examine the toxicopathological effects of this novel class of compounds are needed to more completely evaluate their translational potential.

The SPR analysis showed that α-VE5 and γ-VE5 exhibited selectivity in binding affinity and membrane recruitment for Akt and PHLPP1 compared to other PH domain–containing proteins, such as PDK1 and ILK. However, this selectivity was moderate, as estimated by the ratios of the $K_d$ values, which ranged from 6 to 29, whereas immunocytochemical data revealed a marked difference in the membrane recruitment of the higher-affinity (Akt and PHLPP1) compared to the lower-affinity (PDK1 and ILK) PH domain–containing proteins.
targeted inhibitors (35–37) and has several implications. First, it provides a rationale for the pharmacological exploitation of vitamin E to develop a novel class of Akt inhibitors, of which the proof of principle is provided by α-VE5 and γ-VE5, through side-chain truncation. Second, PTEN mutations, a common genetic aberration in various hereditary and sporadic cancers (38), lead to PIP3 accumulation and subsequent Akt activation. Our finding in LNCaP cells suggests the ability of tocopherol and VE5 to counteract Akt activation secondary to defects such as loss of PTEN function or Scrib dysregulation. Third, targeting the PH domains of Akt and PHLPP1 to facilitate their membrane translocation represents a new concept for developing Akt inhibitors, which warrants investigation.

**MATERIALS AND METHODS**

**Cell lines, culture, reagents, and antibodies**

The prostate cancer cell lines LNCaP and PC-3 were purchased from the American Type Culture Collection and maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) at 37°C in a humidified incubator containing 5% CO2. The LNCaP-abl cells were a gift from Q. Wang (The Ohio State University). Normal prostate epithelial cells were purchased from Lonza and were cultured in the vendor-recommended defined prostate epithelial cell growth medium. For experiments, LNCaP cells were plated on poly-α-lysine–coated culture flasks at a density of 12,000 cells/cm² of surface area for 24 hours, followed by treatment with test agents in serum-free RPMI 1640 medium. α-Tocopherol and γ-tocopherol were purchased from Sigma-Aldrich. The tocopherol derivatives α-VE5 and γ-VE5 were synthesized in the Chen laboratory.

[^2]Orthophosphate was purchased from PerkinElmer Life Sciences. Dioctanoyl PIP2 was obtained from CellSignals Inc. LY-294002 was purchased from LC Laboratories. Antibodies specific to Akt phosphorylated at Ser473, Akt phosphorylated at Thr308, Akt, PDK1, and ILK antibodies were from Cell Signaling Technology Inc.; PHLPP1 antibody was from Novus Biologicals; Na+/K+-ATPase and ILK antibodies were from Santa Cruz Biotechnology; and BTK antibody was from BD Biosciences-Pharmingen. Alexa Fluor 555– and Alexa Fluor 488–conjugated goat anti-rabbit and anti-mouse immunoglobulin G (IgG) antibodies were purchased from Invitrogen, and anti-mouse and anti-rabbit secondary antibodies were obtained from Jackson ImmunoResearch Laboratories.

**Viability assay**

LNCaP cells were plated into poly-α-lysine–coated 96-well plates, and PC-3 and LNCaP-abl cells were plated into uncoated plates at a density of 5000 cells per well in the presence of 10% FBS. Normal prostate epithelial cells were plated into uncoated plates at a density of 8000 cells per well. Exposure to test agents in serum-free medium was initiated 24 hours later. After 24 hours of treatment, cells were incubated with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) (TCl America; final concentration, 0.5 mg/ml) for an additional 2 hours. The medium was then removed from each well and replaced with dimethyl sulfoxide (DMSO) to dissolve the reduced MTT dye for subsequent colorimetric measurement of absorbance at 595 nm. Cell viabilities were calculated as percentages of that in the corresponding vehicle-treated control group.

**Cell lysis and immunoblotting**

Cells were exposed to the test agents in 10-cm dishes for 24 hours and then collected by scraping. The cell pellets were washed once with phosphate-buffered saline (PBS) and then lysed at 4°C over 30 min of incubation in an SDS lysis buffer containing 1% SDS, 50 mM tris-HCl (pH 8.1), 10 mM EDTA, and SigmaFAST protease inhibitor cocktail (Sigma-Aldrich). The cell debris was pelleted by centrifugation for 20 min at 14,000g, and 1 μl of each supernatant was used for determination of protein concentration with a colorimetric bicinchoninic assay (Fierce). The remaining sample was added to equal volume of 2× SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample loading buffer [62.5 mM tris-HCl (pH 6.8), 4% SDS, 5% β-mercaptoethanol, 20% glycerol, 0.1% bromophenol blue], followed by incubation in boiling water for 5 min. Equivalent amounts of total protein were resolved in SDS-polyacrylamide gels and then transferred onto nitrocellulose membranes with a semidry transfer cell. The transferred membrane was washed twice with tris-buffered saline containing 0.1% Tween 20 (TBST). After being blocked with TBST containing 5% nonfat milk for 40 min, the membrane was incubated with the appropriate primary antibody (1:1000) in TBST–1% nonfat milk at 4°C overnight. The membrane was then washed three times with TBST for a total of 15 min, followed by incubation with goat anti-rabbit or anti-mouse IgG–horseradish peroxidase conjugates (1:2000) for 1 hour at room temperature and four washes with TBST for a total of 1 hour. Immunoblots were visualized by enhanced chemiluminescence (GE Healthcare Life Sciences).

**Immunocytochemistry and confocal microscopy**

After 24 hours of treatment in serum-free medium, LNCaP cells were fixated in 4% formaldehyde for 20 min before permeabilization with 0.1% Triton X-100 in PBS at room temperature for 1 hour followed by incubation in 1% FBS (in PBS) for 1 hour. Cells were stained for endogenous PH domain–containing proteins (Akt, PHLPP1, PDK1, ILK, and BTK) by incubation with specific antibodies (1:100), followed by incubation with the Alexa Fluor 555–conjugated goat anti-rabbit or Alexa Fluor 488–conjugated goat anti-mouse IgG (1:2000) at room temperature for 2 hours. Both primary and secondary antibodies were diluted in incubation buffer containing 0.1% Triton X-100 and 0.2% bovine serum albumin in PBS. The cells were washed in PBS after each step and mounted with Vectashield mounting medium supplemented with DAPI (Vector Laboratories Inc.). The slides were allowed to set for at least 4 hours before confocal images were acquired with a Zeiss LSM 510 inverted confocal laser scanning microscope operated with Zeiss LSM 510 software. Image analysis was performed with ImageJ [National Institutes of Health (NIH)] software.

**Coimmunoprecipitation of Akt-PHLPP complexes**

The cytosolic fractions of treated LNCaP cells were isolated as described previously (23) with minor modification. Briefly, cells were resuspended in cytosol buffer [50 mM Hepes (pH 7.4), 10 mM NaCl, 1 mM MgCl2, 0.5 M EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 mM Na3VO4] and then disrupted by 10 passages through a 26-gauge needle. After centrifugation of the cell homogenates at 14,000g for 20 min at 4°C, the supernatants were collected as the cytosolic fractions. Triton-soluble fractions were extracted from the membrane pellets by resuspension in Triton X-100–containing lysis buffer [50 mM tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EGTA, 10 mM MgCl2, 0.5% Triton X-100, and protease inhibitor mixture] and disruption by 10 passages through a 26-gauge needle. After centrifugation of the homogenates at 14,000g for 20 min at 4°C, the supernatants were collected as the Triton-soluble membrane fractions. For coimmunoprecipitation, the Triton-soluble samples were incubated with 30 μl of protein A/G agarose beads for 30 min at 4°C to eliminate nonspecific binding, after which the supernatants were collected by centrifugation at 6000g for 3 min. The samples were then incubated with 10 μl of anti–Akt-bound agarose beads (Santa Cruz Biotechnology) overnight at 4°C. Non-specific IgG was used as a negative control. After brief centrifugation, the beads were collected and washed once with Triton X-100–containing lysis buffer, once with wash buffer 1 [50 mM tris (pH 7.5), 500 mM NaCl, and 0.2% Triton X-100], and once with wash buffer 2 [10 mM tris (pH 7.5), 150 mM NaCl, and 0.5% Triton X-100].
and 0.2% Triton X-100]. Immunoprecipitates were then eluted from the beads by the addition of 70 μl of 2x Laemmli sample buffer, followed by boiling at 95°C for 5 min, and subjected to Western blot analysis.

**Isolation of lipid raft–containing membrane subfractions**

Triton-insoluble membrane constituents were isolated from 5 × 10⁶ treated LNCaP by detergent extraction as described previously (18). Lipid raft–containing membrane subfractions were isolated by centrifugation through sucrose density gradients as described previously (24) with the following minor modifications. After treatment for 24 hours in serum-free medium, 5 × 10⁶ LNCaP cells per treatment group were collected, washed, and disrupted on ice by passage through a 26-gauge needle six times. After centrifugation through a sucrose gradient, consecutive 1.2-ml fractions were collected from the top of the gradient and stored at −20°C until Western blot analysis was performed to assess the abundance of phosphorylated Ser⁴⁷³ in Akt, total Akt, PHLPP1, and flotillin-2 in each fraction.

**Ectopic expression of CA-Akt and siRNA-mediated knockdown of PHLPP1**

Transient transfections were performed with the Amaza Nucleofector System or Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Nucleofection used a commercially available Nucleofector kit (KitR-program T-09) (Lonza Inc.). LNCaP cells were transfected with the plasmid encoding hemagglutinin-tagged CA-Akt (pcDNA-HA-PKB-T308D-S473D) obtained from Addgene. For siRNA-mediated knockdown of PHLPP1, LNCaP cells were transfected with specific siRNA or scrambled siRNA as a control according to the manufacturer's instructions (Dharmacon). The expression of the cloned protein and the knockdown of PHLPP1 were confirmed by immunoblotting.

**Flow cytometry**

For assessment of apoptosis, treated LNCaP cells were stained with annexin V–FITC (fluorescein isothiocyanate) and PI (Invitrogen) according to the manufacturer's protocol. Unstained vehicle-treated control cells, control cells stained with annexin V–FITC only, and control cells stained with PI only were used for gating and background subtraction. For each sample, 10,000 cells were acquired for flow cytometry with a FACSCalibur cytometer (BD Biosciences). Data were analyzed with the FlowJo software program.

**Autoradiographic determination of phosphoinositide formation**

Evaluation of phosphoinositide formation in tocopherol-treated LNCaP cells was performed as described previously (39) with the following modification. After being labeled with [γ-³²P]orthophosphate (HCl-free) in phosphate- and serum-free Dulbecco’s modified Eagle’s medium, the cells were exposed to α-tocopherol for 6 hours in serum-free RPMI 1640 medium.

**Cholesterol measurement**

Aliquots (300 μl) of each of the fractions collected from the sucrose gradient preparation of membrane subfractions were used for measurement of cholesterol concentrations. Each sample was extracted with 300 μl of a chloroform/isopropanol/NP-40 mixture (7:11:0.1). The organic phase was collected by centrifugation at 15,000g for 10 min and then air-dried at 50°C to remove chloroform. Trace amounts of organic solvent were removed by evaporation. The cholesterol content in each sample was analyzed with a Total Cholesterol Assay Kit (Cell Biolabs).

**In vivo study**

Male athymic nude mice (Hsd:Athymic Nude-Foxn1nu/nu, 5 to 7 weeks of age) were purchased from Harlan Laboratories and group-housed under conditions of constant photoperiod (12-hour light/12-hour dark) with ad libitum access to sterilized food and water. All experimental procedures using mice were done in accordance with protocols approved by The Ohio State University Institutional Animal Care and Use Committee. Ectopic tumors were established in athymic nude mice by subcutaneous injection of 1 × 10⁶ PC-3-luc or 2 × 10⁶ LNCaP-abl cells in a total volume of 0.1-ml serum-free medium containing 50% Matrigel (BD Biosciences). The establishment and growth of tumors were monitored weekly by measurement with calipers (tumor volume = width² × length × 0.52) and bioluminescence (PC-3-luc) with the IVIS imaging system (Xenogen Corporation). For bioluminescent imaging, mice anesthetized with isoflurane were imaged at 15, 20, and 25 min after intraperitoneal administration of firefly luciferin (150 mg/kg; Caliper Life Sciences) to capture maximal luminescence. Data acquisition and analysis were achieved with the Living Image software (Xenogen). Mice with established tumors (mean starting tumor volume ± SE: PC-3-luc, 75.3 ± 4.5 mm³; LNCaP-abl, 119.6 ± 7.5 mm³) were randomly assigned to two groups (n = 7 to 8) that received daily intraperitoneal injections of γ-VE5 at 50 mg/kg or vehicle (physiological saline/polyethylene glycol 400/DMSO/Tween 80; 65:20:10:5 by volume) for 21 days. Body weights and tumor burdens were measured weekly. At the study endpoint, mice were euthanized, and blood was collected by cardiac puncture from three mice per group and submitted to The Ohio State University Comparative Pathology and Mouse Phenotyping Shared Resource for determination of complete blood counts and serum chemistry. Tumors were also collected, quickly frozen in liquid nitrogen, and stored at −80°C until analysis of biomarkers.

**Construction of plasmids expressing GFP-tagged Akt and PHLPP1, PH domain of Akt and PHLPP1, and ΔPH-Akt and ΔPH-PHLPP1**

PC-3 cell complementary DNA (cDNA) was used as template for the polymerase chain reaction (PCR) amplification of sequences for the full-length wild-type Akt and PHLPP1 and for the PH domains of Akt and PHLPP1. PCR products were inserted into the pcDNA3.1/CT-GFP-TOPO vector (Invitrogen). To create the GFP-ΔPH plasmids, we amplified the GFP-full-length Akt and PHLPP1-expressing plasmids using primers that omitted the PH domain–coding region of Akt or PHLPP1 in the product. The sequences of the primers used for construction of plasmids are given in table S4.

**Construction of plasmids and expression of GST-PH domain fusion proteins**

The cDNA fragments corresponding to the putative PH domain sequences of PHLPP1 (1115 to +372), PDK1 (11225 to +1671), Akt1 (1+ to +447), and ILK (+385 to +646) were PCR-amplified with the plasmids pCDNA-HA-PHLPP1, pWZL-Neo-Myr-FLAG-PDK1, pcDNA-HA-PKB-T308D-S473D, and pCMV-SPORT-ILK (Addgene) as templates. The PCR products were then cloned into Eco RI/Xho I sites of the pGEX-4T1 expression vector (GE Healthcare Life Sciences) to generate four constructs (pGEX-4T1-PHLPP1, pGEX-4T1PDK1, pGEX-4T1-Akt1, and pGEX-4T1-ILK) for the expression of GST-PH domain fusion proteins. The mutated Akt1 Y388G PH domain was generated from pGEX-4T1-Akt1 by site-directed mutagenesis with the QuickChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies). The expression and purification of GST-PH domain fusion proteins were performed essentially as described previously (27). The purified PH domains were analyzed by SDS-PAGE using an overloaded gel with purities estimated to be >95% using ImageJ software. The sequences of the primers used for construction of plasmids are given in table S4.

**Molecular modeling**

The primary sequence of human Akt (National Center for Biotechnology Information, NP_001014432.1) and the crystal structure of the human...
Akt-PH domain [Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB), 2UZS] were used for the molecular docking simulations. The structures of α-VE5/γ-VE5 were constructed by geometry optimization with CHARMM force field calculation. Docking of α-VE5 or γ-VE5 into the Akt-PH domain was performed with the CHARMM-based molecular docking algorithm implemented in the Discovery Studio 2.1 program (Accelrys Inc.). The flexibility of the compounds was accounted for by including different orientations and rotatable torsion angles in the docking procedure. Accordingly, 108 conformation structures was accounted for by including different orientations and rotatable torsion angles in the docking procedure. Accordingly, 108 conformation structures was accounted for by including different orientations and rotatable torsion angles in the docking procedure. Accordingly, 108 conformation structures was accounted for by including different orientations and rotatable torsion angles in the docking procedure. Accordingly, 108 conformation structures was accounted for by including different orientations and rotatable torsion angles in the docking procedure. Accordingly, 108 conformation structures was accounted for by including different orientations and rotatable torsion angles in the docking procedure.
P. Patrignani, M. R. Panara, S. Tacconelli, F. Seta, T. Bucciarelli, G. Ciabattoni,
D. J. Mulholland, L. M. Tran, Y. Li, H. Cai, A. Morim, S. Wang, S. Plaisier, I. P. Garraway,
C. C. Thomas, M. Deak, D. R. Alessi, D. M. van Aalten, High-resolution structure of
B. S. Carver, C. Chapinski, J. Wongvipat, H. Hieronymus, Y. Chen, S. Chandarlapaty,
M. C. Royer, S. Lemaire-Ewing, C. Desrumaux, S. Monier, J. P. Pais de Barros, A. Athias,
D. R. Alessi, D. M. Van Aalten, (3,4,5)-trisphosphate.

C. C. Milburn, M. Deak, S. M. Kelly, N. C. Price, D. R. Alessi, D. M. Van Aalten,

B. S. Carver, C. Chapinski, J. Wongvipat, H. Hieronymus, Y. Chen, S. Chandarlapaty,
V. K. Arora, C. Le, J. Koutcher, H. Scher, P. T. Scardino, N. Rosen, C. L. Sawyers,

D. J. Muhlolland, L. M. Tran, Y. Li, H. Cai, A. Morim, S. Wang, S. Plaisier, I. P. Garraway,

P. Patrignani, M. R. Panara, S. Taccioni, F. Seta, T. Bucciacocchi, G. Ciabattoni,
F. Alessandrini, A. Mezzetti, G. Santini, M. G. Scili, F. Cioppolone, G. Davi, P. Gallina,


C. Nitsche, M. Edderkaoui, R. M. Moore, G. Eibl, N. Kasahara, J. Treger, P. J. Grippo,
J. Mayerle, M. L. Cher, A. Guvokskyaya, The phosphatase PHLPP1 regulates Akt2,

S. F. Barnett, D. Defeo-Jones, S. Fu, P. J. Hancock, K. M. Haskell, R. E. Jones,
J. A. Kahana, A. M. Kral, K. Leander, L. L. Lee, J. Malinowski, E. M. McAvoy,

D. Mahadevan, G. Powis, E. A. Mash, B. George, V. M. Gokhale, S. Zhang, K. Shakalya,
L. Du-Curry, M. Berggren, M. A. Ali, U. Jana, N. Ihle, S. Moses, C. Franklin, S. Narayan,

S. A. Moses, M. A. Ali, S. Zuophe, L. Du-Curry, L. L. Zhou, R. Lemos, N. Ihle,


Vitamin E Facilitates the Inactivation of the Kinase Akt by the Phosphatase PHLPP1
Po-Hsien Huang, Hsiao-Ching Chuang, Chih-Chien Chou, Huiling Wang, Su-Lin Lee, Hsiao-Ching Yang, Hao-Chieh Chiu, Naval Kapuriya, Dasheng Wang, Samuel K. Kulp and Ching-Shih Chen
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