Therapeutic Discovery

A Novel Human Dynactin-Associated Protein, dynAP, Promotes Activation of Akt, and Ergosterol-Related Compounds Induce dynAP-Dependent Apoptosis of Human Cancer Cells

Tatsuki Kunoh¹, Takanori Noda¹, Koichi Koseki¹, Masayuki Sekigawa¹, Motoki Takagi³, Kazuo Shin-ya⁴, Naoki Goshima⁴, Shun-ichiro Iemura⁴, Tohru Natsume⁴, Shu-ichi Wada¹, Yukio Mukai¹, Shinji Ohta¹, Ryuzo Sasaki², and Tamio Mizukami¹

Abstract

There are several human genes that may encode proteins whose functions remain unknown. To find clues to their functions, we used the mutant yeast defective in Mad2, a component of the spindle checkpoint complex. Phenotypes that were provoked by the expression of a human C18orf26 protein in the mutant yeast encouraged further characterization of this protein in human cells. This protein was designated dynAP (dynactin-associated protein) because of its interaction with dynactin subunits that comprised a microtubule-based motor protein complex. The dynAP is a transmembrane protein localizing to Golgi apparatus and plasma membrane in a microtubule-dependent manner. This protein was expressed in half of human cancer cell lines but barely in normal human fibroblasts tested. The SV40-transformed fibroblasts expressed dynAP. Importantly, the expression of dynAP activated Akt (also known as protein kinase B) by promoting Ser473 phosphorylation required for the full activation, whereas knockdown of dynAP abolished this activation. The ergosterol-related compounds identified by the yeast cell-based high-throughput screen abrogated activation of Akt and induced apoptosis in a dynAP-dependent manner. We propose a possible advantage of dynAP expression in cancer cells; the survival of cancer cells that express dynAP is supported by dynAP-induced activation of Akt, sustaining high rates of proliferation. The inactivation of dynAP by the selected compounds nullifies this advantage, and thereby, the apoptotic machinery is allowed to operate. Taken together, dynAP can be a new target for cancer therapy, and the selected chemicals are useful for developing a new class of anticancer drugs. Mol Cancer Ther; 9(11); OF1–9. ©2010 AACR.

Introduction

As the simplest eukaryotic cells, yeast cells have been used to identify the functions of human proteins and also to screen chemicals that can act as lead compounds for the development of therapeutic agents. Many proteins linked with accelerated proliferation of human cancer cells repress the growth of yeast (1–3). These observations have provided a cell-based high-throughput screening system for anticancer drugs because inhibitors of human proteins can be screened by simply monitoring the restoration of yeast proliferation (4).

The completion of the sequencing of the human genome has revealed the existence of several genes that may encode proteins whose physiologic functions remain unknown. We have undertaken the yeast cell–based screening to find possible functions of human uncharacterized proteins, and >100 proteins have been found to inhibit the growth of the wild-type yeast (5). In the present work, we extended this screening to the mutant yeast defective in Mad2, which is an essential member of spindle checkpoint proteins that monitor the attachment of spindles to kinetochores with sufficient tension to avoid the misseggregation of sister chromatids (6, 7).

We speculate that if the growth of the Mad2-defective yeast is inhibited by expressing a human protein, this protein may be involved in cell proliferation events. Here, we report results that qualify the C18orf26 protein netted by this screening as a novel target for anticancer drugs. Small chemicals that act on this protein and their properties are also presented.

Authors’ Affiliations: ¹Nagahama Institute of Bio-Science and Technology; ²Frontier Pharma, Shiga, Japan and ³Biomedicinal Information Research Center, Japan Biological Informatics Consortium; ⁴Biomedicinal Information Research Center, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan

Note: Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

Corresponding Author: Tamio Mizukami, Nagahama Institute of Bio-Science and Technology, Shiga 526-0829, Japan. Phone: 81-749-64-8169; Fax: 81-749-64-8140. E-mail: mizukami@nagahama-i-bio.ac.jp

doi: 10.1158/1535-7163.MCT-10-0730
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Materials and Methods

Plasmids, cell culture, and transfection

The plasmids used for screening of human cDNAs in budding yeast were constructed with the Gateway cloning system (Invitrogen). Human clones on the entry vector (8) were introduced into pYES-DEST52 (Invitrogen). For expression of green fluorescent protein (GFP) or V5-tagged dynAP in human cells, plasmids based on pcDNA-DEST53 or pcDNA-DEST40 (Invitrogen) were constructed with the same system. The cDNAs of dynAP and Akt1 were subcloned into pFLAG-CMV2 (Sigma) using the standard PCR methods to generate pFLAG-dynAP and pFLAG-Akt1, respectively. To generate pFLAG-Akt1-Neo\(^{\prime}\)/Kan\(^{\prime}\) was generated by the insertion of Neo\(^{\prime}\)/Kan\(^{\prime}\) gene into the Xhol site. The yeast mutant strains were derivatives of the wild-type BY4742 strain constructed by the Saccharomyces Genome Deletion Project. Standard budding yeast techniques and media were used. The human cell lines HCC38, HCT-116, MDA-MB-231, MDA-MB-468, and SH-SY5Y were provided by the American Type Culture Collection. The human cell lines A431, A549, BeWo, CACO-2, DU145, HEK293, HEK293T, Hep G2, HT1080, HUC-Fm, KMST-6, LA-N-1, MKN1, MKN45, MKN74, NIH:OVCAR-3, NB-1, NH-12, PC-3, SK-MEL-28, VA-13, and WI-38 were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. The human cell lines ACHN, Caki-1, CoLo205, DLD-1, Kato III, KB, LNCap, MFF, MCF-7, MKN7, SW480, and WiDr-TC were provided by Cell Resource Center for Biomedical Research. The human cell lines HCC38, HCT-116, and human cell lines A431, A549, BeWo, CACO-2, DU145, HEK293, HEK293T, Hep G2, HT1080, HUC-Fm, KMST-6, LA-N-1, MKN1, MKN45, MKN74, NIH:OVCAR-3, NB-1, NH-12, PC-3, SK-MEL-28, VA-13, and WI-38 were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. The human cell lines ACHN, Caki-1, CoLo205, DLD-1, Kato III, KB, LNCap, MFF, MCF-7, MKN7, SW480, and WiDr-TC were provided by Cell Resource Center for Biomedical Research.

Preparation of cell extracts and immunoblotting

Harvested cells were washed once with PBS (Sigma) and lysed in a lysis buffer (CytoBuster Protein Extract Reagent; Novagen) containing 1 mmol/L phenylmethylsulfonyl fluoride, 10 μmol/L NaF, 10 μmol/L Na3VO4, and a protease inhibitor cocktail (Nacalai Tesque). Cell lysates were incubated on ice for 15 minutes and centrifuged at 13,000 × g for 15 minutes. The following primary antibodies were used: anti-α-tubulin (generously provided by Dr. Andrea Baines, University of Oxford); anti-caspase-8 and anti-poly(ADP-ribose) polymerase (PARP; BD Pharmingen); anti-p150Glued, anti-dynamin, and anti-GM130 (BD Transduction Laboratories); anti-c-myc (Covance); anti-GFP (Roche); anti-FLAG (Sigma); and anti-Akt and anti-phospho-Akt Ser\(^{\text{473}}\) (Epitomics). As secondary antibodies, goat horseradish peroxidase–conjugated mouse or anti-rabbit IgG antibodies (BioSource, ebioscience) were used.

Coimmunoprecipitation

For coimmunoprecipitation, antibodies against c-myc, p150Glued, dynamin, and dynAP or preimmune serum were preincubated with ExtraCruz IP Matrix (Santa Cruz Biotechnology, Inc.) for 2 hours. After three washes, 100 to 500 μg of the cell extracts were added and incubated for another 2 hours. The immunoprecipitates were washed three times and subjected to immunoblotting.

Reverse transcription-PCR

Total RNA was isolated using an RNeasy Mini kit (Qiagen) according to the manufacturer’s protocol.
Reverse transcription-PCR was conducted with ReverTra Ace-α (Toyobo) using 0.8 μg of each total RNA and primers amplifying the full length of the dynAP cDNA. As an internal control, the mRNA of glyceraldehyde-3-phosphate dehydrogenase was detected.

**Short hairpin RNA knockdown of dynAP**

Oligonucleotides described below were annealed and cloned into pcDNA 6.2-GW/EmGFP-miR (Invitrogen). The following primers were used: dynAP_shRNA_1, 5′-TGCTGTGAGCTGCCAGTATCACAAGTTTGGCCACTGACTGACCTTGATGAGCTCAA-3′ (sense) and 5′-CCTGTGGACCTGCCAAATCAAGGTCAGTCAGTGCCAAAACCTTGTGATACATGGCAGCTCAAC-3′ (antisense); dynAP_shRNA_2, 5′-TGCTGTTCACATAGACCATTTTTGGCCCCACTGACTGACATGCTATGGAACAAAACCTTGTGAACTGG-3′ (sense) and 5′-CCTGTGGACATACGACTGTGCTATGGTTTTGGCAGCTCAAC-3′ (antisense); dynAP_shRNA_3, 5′-TGCTGTGGAAGTTGCTAGTGGTTTTGGCCTTGACTGACCTACATGGCATACATGGCAACTCAATAGGACACCATCCACTCAAC-3′ (sense) and 5′-CCTGTGGACATTTGGCAAGTTGCTATGGTTTTGGCAGCTCAAC-3′ (antisense).

Cell extracts of HeLa transfected with resultant plasmids pdynAP_shRNA_1-3 or the control vector (pcDNA 6.2-GW/EmGFP-miR-neg control) were subjected to immunoblotting. Unfortunately, the reduction of dynAP expression was observed only when pdynAP_shRNA_1 was introduced into HeLa cells (data not shown). Therefore, further experiments were done by using pdynAP_shRNA_1.

**Immunofluorescence microscopy**

Immunostaining of yeast cells was carried out as described previously (9). Human cells grown on poly-L-lysine–coated coverslips were fixed in 70% methanol or 3% formaldehyde for 10 minutes, followed by blocking and permeation in PBS containing 1% bovine serum albumin and 0.1% Triton X-100 (Nacalai Tesque) for 30 minutes. For the nonpermeabilized condition, Triton X-100 was omitted from the buffer. The cells were then incubated with antibodies against α-tubulin, β-tubulin (Lab Vision), FLAG, and GM130. As the secondary antibody, Cy3-conjugated donkey anti-rabbit or anti-mouse antibodies (Jackson ImmunoResearch) were used. For staining of V5-labeled dynAP, the Cy3-conjugated anti-V5 antibody was used. Images were acquired with an Axioskop 2 plus microscope (Carl Zeiss).

**Flow cytometry**

After gentle trypsinization, cells were centrifuged into a pellet and fixed in ice-cold 70% ethanol. After removal of the ethanol, the cells were resuspended in PBS containing 2.5 μg/mL propidium iodide and 0.5 mg/mL RNaseA (Nacalai Tesque) and analyzed using a JSAN Cell Sorter (Bay Bioscience).

**Survey of proteins interacting with dynAP**

The cellular proteins that might interact with dynAP were surveyed using the previously reported method (10). Briefly, extracts prepared from HeLa and HEK293T cells transiently expressing FLAG-tagged dynAP were immunoprecipitated using an anti-FLAG antibody, followed by elution with the FLAG peptide. After digestion of the eluted proteins with a Lys-C endopeptidase, the resulting peptides were analyzed using a highly sensitive direct nanoflow liquid chromatography/tandem mass spectrometry method.

**Results**

**Screening with the mutant yeast**

Approximately 10,000 human cDNAs under the control of an inducible GALT promoter were introduced into both the wild-type yeast and the mutant yeast lacking Mad2. Among several human proteins that inhibited the growth of the mutant yeast but not the wild-type yeast, C18orf26 protein, which we dubbed “dynAP,” repressed the growth of mutant yeasts deficient in other spindle checkpoint factors, Mad1, Mad3, and Bub3 (Supplementary Fig. S1A). In addition, expression of dynAP produced abnormally elongated spindles not only in the mutant yeast but also in the wild-type yeast (Supplementary Fig. S1B). These long spindles may generate a signal(s) repressive of cell proliferation. Presumably, the wild-type yeast overcomes the signal but the mutant yeast is unable to do so. The molecular mechanism that represses growth of the mutant yeast is currently unknown. Nevertheless, this finding prompted us to study dynAP in human cells because the formation of abnormal spindles suggested that dynAP was somehow involved in microtubule dynamics.

**Predicted properties of dynAP**

The nucleotide sequence of the dynAP cDNA revealed a possible mRNA encoding a protein of 210–amino acid residues with a molecular mass of 22.5 kDa. This putative protein contains a potential transmembrane domain (amino acids 113–133) and a threonine-rich domain (amino acids 172–207; Supplementary Fig. S2A). An alignment of the amino acid sequence indicated that these domains were highly conserved among mammals (Supplementary Fig. S2B) but not in yeasts, flies, and nematodes.

**Expression of dynAP in human cells**

The expression of dynAP was examined using a polyclonal antibody raised against a peptide in dynAP (amino acids 19–32). The protein with 45 kDa was detected in the extracts of HeLa cells (Fig. 1A, left). When GFP-labeled dynAP was stably expressed and the anti-dynAP antibody was used for immunoblotting, two bands with 45 and 70 kDa were found. When anti-GFP antibody was used, only GFP-labeled dynAP with 70 kDa was detected.
Knockdown of dynAP with short hairpin RNA (shRNA) caused almost complete disappearance of 45-kDa band (see Fig. 3C), confirming that the protein with 45 kDa was the endogenous dynAP. The size of dynAP is much larger than the size of 22.5 kDa calculated from its amino acid sequence. The recombinant dynAP expressed in insect SF9 cells migrated in SDS-polyacrylamide gel with a size of 30 kDa (data not shown), suggesting mammalian cell-specific posttranslational modification(s).

Among 40 human cancer cell lines tested, 19 cell lines (48%) including ACHN and HeLa expressed a distinct dynAP of 45 kDa (Supplementary Fig. S3A). dynAP was abundantly expressed in ACHN, whereas its expression in HeLa cells was relatively low. The expression in other 21 cell lines including DLD-1 was almost undetectable. In agreement with the immunoblotting analyses, expression of dynAP mRNA was intense in ACHN and Caki-1 cells, but far less in HeLa cells and almost undetectable in DLD-1 cells (Supplementary Fig. S3B). In two normal fibroblast cell lines (HUC-fm from umbilical cord and WI-38 from lung), the expression of dynAP was very low (Fig. 1B). Interestingly, the substantial expression was observed in WI-38 cells transformed with SV40 (WI-38VA-13 in Fig. 1B). The increased expression of dynAP in the SV-transformed cells was correlated well with an increase of its mRNA (Supplementary Fig. S3B).

**Subcellular localization of dynAP**

When GFP-labeled dynAP was expressed in HeLa cells, the intense signal was found in the plasma membrane (Fig. 1C). Furthermore, localization of the labeled dynAP overlapped with that of GM130, a Golgi marker protein (Fig. 1C, top). It is of interest that dynAP is most densely concentrated at cell-cell borders (Fig. 1C, bottom). Biochemical analyses indicated that dynAP was mainly concentrated in the membrane fraction (data not shown). In addition, removal of the putative transmembrane domain from dynAP caused a diffuse localization of this protein in cytoplasm (Supplementary Fig. S4A). From these results, we conclude that dynAP is a transmembrane protein predominantly localizing to Golgi apparatus and plasma membrane. The membrane topology of dynAP was determined by immunostaining of HeLa cells expressing the V5-tagged version at COOH terminus and FLAG-tagged version at NH2 terminus with the anti-V5 and anti-FLAG antibodies, respectively (Supplementary Fig. S4B). Consistent with the localization of GFP-dynAP (see Fig. 1C), both V5- and FLAG-labeled dynAPs in the...
permeabilized cells were found in the plasma membrane and Golgi apparatus. In the nonpermeabilized conditions under which the antibodies were inaccessible to the intracellular dynAP, immunofluorescent signal of dynAP-V5 was detected only in the plasma membrane, whereas that of FLAG-dynAP was hardly detected anywhere. Thus, the NH₂-terminal region of dynAP is exposed to the cytoplasmic side. Localization of dynAP to Golgi apparatus and plasma membrane was lost after culture with nocodazole, a microtubule depolymerizer (Supplementary Fig. S4C), indicating that the intact microtubules are required for the relevant localization of dynAP.

Identification of proteins that interact with dynAP

Immunoprecipitates from HeLa and HEK293T cells expressing FLAG-tagged dynAP were analyzed to examine proteins associated with dynAP (Supplementary Tables S1 and S2). Among candidates whose interactions with dynAP were suggested in both cell lines, dynamitin gained our attention, because dynamitin is related to microtubules and the expression of dynAP in yeast produced elongated spindles (see Supplementary Fig. S1B). Dynamitin (also known as dynactin 2 or p50) is a major component of the dynactin complex (11). Dynactin and dynein form a microtubule-based motor protein complex...
that is responsible for transporting intracellular cargos, microtubule stability, and positioning of Golgi apparatus (12–14). Physical interactions of dynAP with two dynactin components (dynamitin and p150Glued) were confirmed immunochemically in ACHN cells (Fig. 2A–C) and in several human cancer cells that expressed dynAP abundantly (Supplementary Fig. S5).

**dynAP promotes activation of Akt**

In the course of surveying signaling pathways that were affected by overexpression of dynAP, we found that the serine-threonine kinase Akt (also known as protein kinase B) was activated. Overexpression of dynAP in HeLa cells increased the phosphorylated form of Akt at Ser473 (phospho-Ser473; Fig. 3A). Phosphorylation of this site is required for the full activation of Akt (15). The activated form of Akt was undetectable in the normal fibroblasts (WI-38) that did not express dynAP, whereas the activated form was found at a significant level in the SV40-transformed counterpart (VA-13) that expressed dynAP (Fig. 3B). Knockdown of dynAP with shRNA markedly attenuated phosphorylation of Akt at Ser473 (Fig. 3C). Finally, the active form of Akt completely disappeared when HeLa cells overexpressing dynAP and ACHN cells were treated with LY294002 (Fig. 3D). This compound was thought to be an inhibitor specific for phosphatidylinositol 3-kinase (PI3K), but more recently, the compound has been shown to block rictor-mTOR (mammalian target of rapamycin) that phosphorylates Ser473 of Akt (16). Although we have not attempted to identify the target of this compound in the present work, our results are sufficient to conclude that dynAP promotes activation of Akt. Because involvement of the Akt signaling pathway in tumorigenesis and/or tumor progression has been amply documented (17, 18), we decided to search the chemicals that act on dynAP by the use of yeast cell-based screening.

**Screening of chemicals that reverse the dynAP-dependent repression of yeast growth**

The broths prepared from fungal and bacterial cultures were screened for the chemicals that restored proliferation of Mad2-deficient cells expressing dynAP. Isolation and

![Figure 4](https://mct.aacrjournals.org/)

Figure 4. Screening of chemicals that reverse the dynAP-dependent repression of growth of the mutant yeast. A, structural formulas of the identified compound, 22,23-epoxy-3,12,14,16-tetrahydroxy-ergosta-5,7-dien-11-one (compound 5), and its analogous compounds (1–4 and 6–8) used in this study. B, exponentially growing Δmad2 mutant cells harboring the empty vector or pdynAP were washed and adjusted for their cell density, followed by incubation in galactose medium containing the indicated concentrations of compounds 1 to 8. After 48 h, the growth recoveries were calculated by measuring the absorbances (A) of the cell cultures.
structural analyses of the selected compounds were described elsewhere (19). The most effective hit was a novel ergosterol-related compound, \((\beta_3,12\beta,14\beta,16\beta)-22,23\)-epoxy-3,12,14,16-tetrahydroxyergosta-5,7-dien-11-one, designated compound 5 (Fig. 4A and B). A further study of the compound 5 analogues revealed that the compounds 4, 7, and 8 recovered the growth of the mutant yeast repressed by dynAP expression (Fig. 4B).

**Selected chemicals induce dynAP-dependent apoptosis of human cancer cells and abrogate activation of Akt**

Subsequently, the effects of eight selected chemicals on human cancer cells were examined. Culture of ACHN cells with compounds 4 and 8 severely blocked cell proliferation, whereas that with the other compounds had no effects (Fig. 5A). Flow cytometry revealed that the sub-G1 fraction, an index of fragmented DNA in apoptosis, increased significantly on culture with compounds 4 and 8 but not with other compounds (Fig. 5B; Supplementary Fig. S6A). Cleavage of procaspases and PARP is the molecular hallmark of apoptotic cell death (20). Treatment of ACHN cells with either compound 4 or compound 8 caused the cleavage of caspase-8 and PARP, whereas other compounds did not (Fig. 5C).

ACHN, HeLa, and DLD-1 expressed dynAP in the different levels (high, low, and very low levels, respectively; see Supplementary Fig. S3A). To examine whether the compound 4–induced apoptosis was dependent on the dynAP levels, these cells were treated with compound 4. The sub-G1 fraction in ACHN cells increased at a low compound 4 dose (5 \(\mu\)mol/L), whereas in DLD-1 cells remained very low at a high dose (25 \(\mu\)mol/L; Fig. 6A; Supplementary Fig. S6B). HeLa cells were intermediate between ACHN and DLD-1 cells with respect to the sensitivity to compound 4, correlating well with the dynAP levels. Likewise, a close correlation between the dynAP level and susceptibility to compound 4 was also found by detecting cleavage of caspase-8 and PARP (Fig. 6B). The undetectable cleavage of caspase-8 in DLD-1 cells was not due to a lack of the apoptotic pathway because the staurosporine-induced cleavage of caspase-8 was observed in DLD-1 cells (data not shown). Overexpression of GFP-dynAP facilitated compound 4–induced cleavage of caspase-8 (Fig. 6C). The time course experiments with compound 4 indicated that cleavage of PARP and caspase-8 in ACHN cells occurred earlier than that in HeLa cells (Supplementary Fig. S7A), consistent with the notion that an increase in the dynAP level makes cells more susceptible to compound 4. This was also true for the human fibroblasts; cleavage of caspase-8 on treatment with compound 4 was almost undetectable in the normal fibroblast cell lines (HUC-fm and WI-38) that barely expressed dynAP, whereas the cleavage was clearly seen in the SV40-transformed WI-38VA-13 cell line that expressed dynAP (Fig. 6D).

dynAP increased the level of activated Akt (see Fig. 3A), and compound 4 induced apoptosis in a dynAP-dependent manner (see Fig. 6A–D). Therefore, it is important to examine the effect of compound 4 on the activation of Akt. Treatment of HeLa and ACHN cells with compound 4 caused almost complete disappearance of the activated Akt (Fig. 6E). In HeLa cells stably expressing FLAG-Akt, the level of the activated Akt markedly increased (Supplementary Fig. S7B). Treatment of these clones with compound 4 decreased the activated Akt in a dose-dependent manner, whereas the total Akt level did not change significantly. The decrease in the activated Akt well correlated with cleavage of PARP and caspase-8. These results have led us to propose that the compound 4–induced inactivation of Akt plays a key role in the induction of apoptosis by the selected chemicals.
of dynAP impedes the activation of Akt that acts as an anti-apoptotic signal, and thereby induces apoptotic processes.

Discussion

Serine/threonine Akt kinase is a key regulator of a wide range of cellular pathways including growth, proliferation, and survival. Accordingly, deregulation of Akt plays a key role in the onset or propagation of cancer. We found that dynAP is a novel modulator of Akt activation, warranting dynAP as a potential target for developing anticancer drugs. In addition, the involvement of Akt in the metabolic control (lipid and glucose) has been widely accepted, and recently, the importance of Akt has been implicated in the neuronal function (17, 18). Expression of dynAP therefore may profoundly affect other disease conditions such as diabetes mellitus and neurodegenerative diseases. The cellular content of dynAP seemed to be correlated with its mRNA level, and SV40 transformation provoked dynAP expression in the normal fibroblasts that did not express dynAP. These results may suggest that the expression of the dynAP gene is under the control of a tumor suppressor (Rb or p53) at the transcriptional level, although the control may be indirect because the canonical sequences responsive for these transcription factors are not found in the dynAP gene. Exploration of tissue-specific dynAP expression including cancer tissues and clarification of regulatory mechanism of its gene expression are essentially required for understanding the roles of this protein in physiologic processes as well as in pathogenesis of cancers, which is currently under way.

Akt is the central effector of PI3K; the Akt activity is induced by the activation of PI3K in growth factor receptor-mediated signaling cascades. Activation of PI3K increases 3′-phosphoinositides, PI(3, 4)P2 and PI(3,4,5)P2, to which Akt binds through its pleckstrin homology domain. This binding is thought to impose conformational changes in Akt, allowing Ser473 in the hydrophobic motif to be phosphorylated. Phosphorylation of this site is required for the full activation of Akt. Rapamycin-insensitive mTORC2 (rictor-mTOR) has recently been identified as a Ser473 kinase (16), although involvement of other kinases has not been precluded (17, 18). dynAP promoted Ser473 phosphorylation of Akt. This promotion can be achieved through several ways: activation of PI3K or mTOR, inhibition of PTEN (a phosphatase for 3′-phosphoinositides)
or PHLPP (a phosphatase for Akt phospho-Ser473), and an interacting partner–induced conformational change of Akt by which Ser473 is phosphorylated with a high efficiency. Complete disappearance of Akt phospho-Ser473 by LY294002, an inhibitor of PI3K and mTOR, made it unlikely that PTEN or PHLPP was involved. It was recently reported that peptidyl-prolyl isomerase Pin1 is critical for Akt stability and phosphorylation for its activation (21). Intriguingly, physical interaction of dynAP with peptidyl-prolyl isomerase was found in two types of cell lines (HeLa and HEK293T; see Supplementary Tables S1 and S2). Whether Pin1 is involved in the dynAP-induced phosphorylation of Akt or not needs further studies.

Our ergosterol-related compound 4 almost completely abolished the activated form of Akt and induced apoptosis of the cells that expressed dynAP. Conceivably, the expression of dynAP supports the survival of cancer cells through activation of Akt and thereby sustains high rates of proliferation. The compound 4–induced inactivation of dynAP abolishes the activation of Akt, resulting in the operation of apoptotic machinery. Although compelling evidence has yet to be obtained, it is very likely that compounds 4 and 8 interact directly with dynAP because they specifically act on human cells that express dynAP.

We have undertaken yeast cell–based screening to find novel functions of human uncharacterized proteins, and >100 proteins have been found to inhibit the growth of wild-type yeast (5). dynAP was not positive in this screen because of the little effect on growth of the wild-type yeast (5). dynAP was not positive in this screen.

In short, the yeast-based screening is very promising for finding clues that suggest functions of human uncharacterized proteins for which neither the in vitro assay nor the target-oriented cell-based assay can be constructed. We anticipate that the small molecules obtained by the high-throughput functional screen will provide novel strategies for the treatment of diseases and also greatly expedite studies of biological functions of these proteins.

Disclosure of Potential Conflicts of Interest

T. Mizukami received a commercial grant from, has ownership interest in, and is a consultant/on advisory board of Frontier Pharma.

Acknowledgments


Grant Support

New Energy and Industrial Technology Development Organization and Frontier Pharma.

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Received 08/05/2010; revised 09/08/2010; accepted 09/14/2010; published OnlineFirst 10/26/2010.

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Molecular Cancer Therapeutics

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Mol Cancer Ther  Published OnlineFirst October 26, 2010.

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doi:10.1158/1535-7163.MCT-10-0730

Supplementary Material
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