RhoA-dependent Murine Prostate Cancer Cell Proliferation and Apoptosis: Role of Protein Kinase Cζ

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ABSTRACT

We previously showed that RhoA played an important role in the proliferation of murine Waprostate cancer (TRAMP) cells (P. M. Ghosh et al., Oncogene, 18: 4120–4130, 1999). Untransformed TRAMP cells as well as those expressing constitutively active RhoA (Q63L) mutant protein (Q63L cells) were highly proliferative. In contrast, TRAMP cells expressing dominant-negative RhoA (T19N) mutant protein (T19N cells) were slow growing. In this study, we showed, in addition, that T19N cells displayed reduced rates of apoptotic cell death in response to serum deprivation, compared with TRAMP and Q63L cells, and we studied the mechanisms of the effects of RhoA on TRAMP cell proliferation and apoptosis. Both proliferation and apoptosis of TRAMP and Q63L cells were dependent on the activation of phosphatidylinositol 3-kinase (PI3K). The ubiquitous mitogen-activated Ser/Thr kinase, p70S6 kinase, a downstream effector of PI3K, was overexpressed in TRAMP and Q63L cells. Another PI3K effector, the cell survival protein Akt, displayed increased activity in T19N cells, which did not express active RhoA, compared with TRAMP and Q63L cells. The atypical protein kinase Cζ (PKζCζ) isofrom PKζCζ, which is downstream of PI3K, was activated in cells expressing active RhoA. In addition, expression of constitutively activated PI3ζCζ in TRAMP cells enhanced proliferation and p70S6 kinase phosphorylation, whereas the inhibition of PKζCζ activation resulted in increased activation of Akt and enhanced cell survival. Thus, the effects of RhoA on TRAMP cell proliferation and apoptosis may be mediated by PKζCζ.

INTRODUCTION

RhoA small GTPase is a member of the Ras superfamily of small GTPases. RhoA mediates the formation of actin stress fibers and focal contact formation, and, thus, plays an important role in cell motility (1). Activation of RhoA is also necessary for cell cycle progression (2, 3). Several investigators have shown that RhoA activation is necessary for normal cell growth as well as for maintaining the transformed phenotype (4–6). Avraham and Weinberg (7) suggested that RhoA is a proto-oncogene because amplification of the human RhoA gene in NIH 3T3 cells resulted in cells that were tumorigenic in nude mice. Also, overexpression of RhoA has been found to promote the invasive potential of cultured rat hepatoma cells (8). Although there have been no reports of genetic alterations in the RhoA gene in human cancers (9), RhoA has frequently been found to be overexpressed in a variety of human tumors (8).

Stimulation of cell proliferation takes place by the transmission of signals relayed by growth factors from membrane-bound receptor Tyr kinases to the nucleus through a series of protein cascades that require serial phosphorylation at Tyr or Ser/Thr sites (10). The two best-known signaling cascades involved in the transmission of these signals are the MAPK3 and the PI3K pathways. Activation of receptor Tyr kinases results in the activation of p21Ras (11, 12). In turn, Ras activates Raf, which then activates MAPK kinase (MEK), resulting in p42/p44MAPK [also known as extracellular regulated kinase (ERK1/2)] phosphorylation (13). PI3K is an enzyme that participates in a myriad of cellular processes and whose activity has been linked to cell growth and transformation, differentiation, motility, insulin action, and cell survival (14). PI3K comprises a family of two-subunit (regulatory p85 and catalytic p110) lipid signaling enzymes that initiate signaling cascades by generating three distinct membrane lipids, includingPIP3 (15). PIP3 phosphorylates and activates PDK-1, which mediates most of the effects of PI3K (16).

One downstream effector of PI3K, the ubiquitous mitogen-activated Ser/Thr kinase, p70S6 kinase, has emerged as an important regulator of cell growth. It participates in the translation of mRNAs that contain an oligopyrimidine tract at the transcriptional start site. The regulation of p70S6 kinase includes phosphorylation at multiple sites. Thr 229, which is located in the catalytic loop of p70S6 kinase, is phosphorylated in a PI3K-dependent manner by PKD1. p70S6 kinase is also activated in vivo in part, by a phosphatidylinositol kinase-related kinase, mTOR (17–19). The immunosuppressant drug rapamycin inhibits mTOR by forming a stable complex with FK506-binding protein, which binds mTOR. As a result of this interaction, rapamycin induces dephosphorylation of several sites of p70S6 kinase, leading to its inactivation (17). The cell survival protein, Akt/PKB, is another downstream effector of PI3K that has been extensively studied. PKB/Akt is controlled at the level of protein kinases (PDK1 and ILK), that by themselves are stimulated in the presence of PIP2 and PIP3 (20). Activation of Akt has been implicated in protection from apoptosis in response to several signals (reviewed in Ref. 20).

A third class of protein kinases regulated by PI3K are the members of the PKC superfamily. The PKC superfamily is comprised of Ser/Thr protein kinases found in mammalian cells; these distinct isofoms of PKC were implicated in a multitude of cellular processes (21). PKCs are subdivided into three subfamilies according to their lipid-activation profiles: conventional PKCs (α, β1, βII, and γ) are activated by DAG and Ca2+; novel PKCs (δ, ε, η, and θ) do not respond to Ca2+ but require DAG; and atypical PKCs (ζ, μ, and λ/δ) are not activated by either DAG or Ca2+ (22). PI3K activates all isofoms of PKCs (23). Phosphorylation of PKC is an important mechanism for regulating its activity (24).

PKζCζ, an atypical family member, has received considerable attention as a downstream target of PI3K. PKD-1 phosphorylates and activates PKζCζ in a PIP3-enhanced manner (25). Stimulation of cells by insulin and insulin-like growth factor leads to activation of PKζCζ, which can be inhibited by chemical inhibitors of PI3K (26). The closely related PKCA isoform is activated in cells by epidermal growth factor or platelet-derived growth factor by a PI3K-dependent

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3 The abbreviations used are: MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol 3,4,5-trisphosphate; mTOR, mammalian target of rapamycin; PKC, protein kinase C; PI, propidium iodide; PDK-1, 3-phosphoinositide-dependent kinase-1; DAG, diacylglycerol.
mechanism (27). These findings implicate atypical PKCs as effectors of PI3K.

We previously reported the importance of RhoA in the growth of prostate tumor cells derived from transgenic mice with adenocarcinoma of the prostate (TRAMP cells; Ref. 3). TRAMP cells expressing dominant-negative RhoA (T19N) mutant protein (T19N cells) displayed few actin stress fibers and grew at a slower rate than control cells because of a lengthening of the G1 phase of the cell cycle. On the other hand, TRAMP cells expressing constitutively active RhoA (Q63L) mutant protein (Q63L cells) displayed a contractile phenotype and grew at a faster rate than controls because of a shorter G1 phase of the cell cycle (3). In this article, we show that RhoA activation results in activation of a p70S6 kinase-dependent cell proliferation pathway. In contrast, inhibition of RhoA activation results in the activation of Akt and enhanced cell survival. RhoA activation resulted in phosphorylation (activation) of PKCζ, whereas inactivation of PKCζ induced Akt phosphorylation, which indicates a role for PKCζ in RhoA-dependent inactivation of Akt.

MATERIALS AND METHODS

Materials. PD 98059, LY 294002, and rapamycin were purchased from Calbiochem, San Diego, CA. The following antibodies were used in the Western blotting experiments: AKT1/2 and p70S6 kinase were from Santa Cruz Biotechnology, Santa Cruz, CA; phospho-Akt1 (Ser473) and phospho-p70S6 kinase (Thr421/Ser424) were from Cell Signaling Technology, Beverly, MA. Phospho-PKCζ (Thr410) was a generous gift from Dr. Alex Toker, Harvard Medical School, Boston, MA (28). Total PKCζ was purchased from Upstate Biotechnology, Lake Placid, NY.

Cell Culture. Transgenic mouse prostate (TRAMP) cancer cells were kindly supplied by Dr. Norman Greenberg, Baylor College of Medicine, Houston, TX, and are a clonal derivative of the TRAMP-C2 cell line (29). This kind was provided by Dr. Norman Greenberg, Baylor College of Medicine, Houston, TX, and are a clonal derivative of the TRAMP-C2 cell line (29). This kind was provided by Dr. Norman Greenberg, Baylor College of Medicine, Houston, TX, and are a clonal derivative of the TRAMP-C2 cell line (29).

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RESULTS

RhoA-stimulated TRAMP Cell Proliferation Is Mediated by PI3K. We previously showed that RhoA activation stimulates proliferation of murine prostate cancer (TRAMP) cells (3). In this study, we used untransfected TRAMP cells as well as those stably transfected with constitutively activated RhoA cDNA (Q63L cells) or dominant-negative RhoA cDNA (T19N cells) to determine whether RhoA-induced cell growth was stimulated via the PI3K or MAPK signaling pathways. TRAMP, Q63L, and T19N cells were treated with and without PD 98059 (20 μM), an inhibitor of MAPK kinase (also called MEK), or the PI3K inhibitor LY 294002 (25 μM). Cell cycle progression was measured by flow cytometry. Fig. 1 illustrates the rate of proliferation of control, Q63L, and T19N cell lines using the percentage of cells in the S phase of the cell cycle as the proliferative index. T19N cells displayed a 50% decrease in proliferation rate compared with parent cells (Fig. 1) and were further growth-inhibited after treatment with both PD 98059 (26% decrease as compared with untreated T19N cells), and LY 294002 (38% decrease as compared with untreated T19N cells). In contrast, treatment of TRAMP and Q63L cells with LY 294002 displayed a 50% decrease in the percentage of cells in S phase of the cell cycle; a much smaller effect (6–12% decrease) was observed with PD 98059 (Fig. 1). Decreased cell proliferation was attributable to G1-G0 arrest (Fig. 1). This suggested the activation of a PI3K-dependent proliferation pathway in cells expressing active RhoA. To verify the results obtained with LY 294002, we stably transfected TRAMP cells with a plasmid expressing a dominant-negative mutant of the p85 (catalytic) subunit of PI3K (Δp85α). The Δp85α-transfected cells (T-Δp85α) decreased Tyr phosphorylation of p85Δ53, and decreased phosphorylation of p70S6 kinase and Akt, two prominent downstream targets of PI3K (Fig. 2A). Expression of Δp85α in TRAMP cells reduced the proliferation rate of these cells by about 75% (Fig. 2B).
To determine whether p70S6 kinase plays a role in RhoA-mediated TRAMP cell proliferation, we inhibited p70S6 kinase activation in these cells with rapamycin. Activation of the mTOR is necessary for p70S6 kinase phosphorylation (activation) along with PI3K (32). The

Fig. 1. Change in the proliferative rate of TRAMP cells under conditions of RhoA activation or inactivation. TRAMP cells stably transfected with constitutively active (Q63L) or dominant-negative (T19N) RhoA mutant plasmids or the empty vector (cont) were treated with LY 294002 (LY, 25 μM), PD 98059 (PD, 20 μM), or DMSO alone, for 24 h, and the percentage of cells in S phase of the cell cycle was determined by flow cytometry. The percentage of cells in S phase for each condition was plotted to express the relative changes in proliferation with respect to controls (proliferative index). The percentage of cells in the G0/G1 phase of the cell cycle was also determined and is given in parentheses for each condition. The data represent the mean of six different experiments. The T19N cells proliferate more slowly compared with TRAMP and Q63L cells. LY 294002, but not PD 98059, inhibited TRAMP and Q63L cell proliferation. On the other hand, both PD and LY inhibited T19N cell proliferation.

TRAMP CellsExpressing Inactive RhoA Displayed Increased Cell Survival. Next, we determined whether RhoA affects cell survival in these cells. TRAMP, Q63L, and T19N cells were deprived of serum for 6 h, in the presence or absence of PD 98059 (20 μM) or LY 294002 (25 μM). The number of apoptotic cells was determined by flow cytometry after staining with Annexin V and PI (Ref. 30; Fig. 3). T19N cells were significantly protected from apoptosis induced by serum deprivation compared with TRAMP and Q63L cells (5% apoptotic T19N cells versus 10% TRAMP and 18.6% apoptotic Q63L cells). Inhibition of the PI3K signaling pathway with LY 294002 resulted in increased apoptosis in T19N cells after serum withdrawal (24.7 versus 5% in untreated cells). PD 98059 induced apoptosis in T19N cells, but not in the cells expressing active RhoA (Fig. 3).

Cells Expressing Active RhoA Demonstrated Enhanced Expression of p70S6 Kinase. To determine the events downstream of PI3K activation resulting in RhoA-mediated apoptosis and proliferation, we examined p70S6 kinase, a downstream effector of PI3K, previously shown to regulate proliferation (31). p70S6 kinase displayed enhanced phosphorylation and overexpression in the Q63L cells as compared with controls as determined by immunoblotting with a phospho-specific p70S6 kinase antibody (ser424/thr421; Fig. 4A). The T19N cells, on the other hand, displayed enhanced phosphorylation and overexpression in the Q63L cells, but not in T19N cells.

Fig. 2. A, immunoblots demonstrating the expression of a dominant-negative PI3K (Δp85α) cDNA in TRAMP cells. Top panels, cell lysates from control- and Δp85α-transfected cells (T-Δp85α) were immunoprecipitated with anti-p85α antibody and the immunoprecipitates run on a 6% SDS-page. The immunoblot was stained with antiphosphotyrosine antibody to detect phosphorylated (activated) p85. The transfected cells expressed decreased Tyr phosphorylation (activation) of p85. The same blot was then stripped and re-stained with anti-p85 antibody. This experiment was repeated three times with similar results. Middle and bottom panels, to ensure that decreased p85 phosphorylation indicated diminished activity, we also stained immunoblots to detect the presence of phosphorylated (activated) p70S6 kinase and Akt, two prominent downstream effectors of PI3K. The T-Δp85α cells also expressed decreased levels of phospho-p70S6 kinase and phospho-Akt, indicative of decreased PI3K activity. Blots were stripped and stained with antibodies to total proteins as a loading control. B, flow cytometric analysis of control- and Δp85α-transfected TRAMP cells to demonstrate that the inhibition of PI3K activation inhibits cell proliferation. The proliferative rates were compared by determining the percentage of cells in S phase normalized to controls (see “Materials and Methods” for details).

Fig. 3. Percentage of apoptotic cells as detected by staining with Annexin V. Apoptosis was induced in these cells by serum starvation for 6 h. The cells were then collected by trypsinization and stained with Annexin V. Stained cells were sorted by flow cytometry (see “Materials and Methods” for details). Annexin V-stained cells were apoptotic, whereas unstained cells were deemed viable. T19N cells were protected from apoptosis induced by serum deprivation compared with TRAMP and Q63L cells. Treatment with the PI3K inhibitor, LY (25 μM), increased the percentage of apoptotic cells. In T19N cells but not in the other cell lines, PD 98059 also induced apoptosis. The data represent the mean of four experiments.

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immunosuppressant rapamycin (100 nM), a specific inhibitor of mTOR, inhibited p70S6 kinase activation identical to LY 294002 (not shown) and inhibited cell cycle progression by ~50% both in the presence and absence of active RhoA (Fig. 4C). This demonstrates the essential role of p70S6 kinase activity for TRAMP cell proliferation. On the other hand, rapamycin did not significantly affect the apoptotic rate of TRAMP cells (not shown).

RhoA Negatively Regulates the Cell Survival Protein Akt. The PI3K-dependent protein kinase Akt (also known as PKB) has been identified as a prominent antiapoptotic protein. Akt was examined by immunoblotting with a phospho-specific antibody against Akt (Ser473). Although the T19N cells demonstrated a high level of activation of Akt, Akt phosphorylation in the TRAMP and Q63L cells was suppressed, which indicated that the expression of active RhoA suppresses the activation of a cell survival pathway (Fig. 5A). Activation of Akt was dependent on PI3K activation as demonstrated by the reduced phosphorylation of Akt with LY 294002 treatment, but not with PD 98059 treatment (Fig. 5B). The activation of p70S6 kinase in the presence of active RhoA, and the activation of Akt in its absence, suggests that PI3K is activated both in the presence and the absence of active RhoA.

The Effects of RhoA on Proliferation and Survival May Be Mediated by PKCζ. We also examined the effects of RhoA on the activation of the atypical PKC isoform PKCζ, another downstream effector of PI3K (28). Immunoblots of TRAMP, Q63L (expressing constitutively active RhoA), and T19N (expressing dominant-negative RhoA) cells were stained with a phospho-specific antibody to PKCζ (Thr410). As can be seen in Fig. 6A, T19N cells expressed decreased levels of phosphorylated PKCζ compared with TRAMP and Q63L cells. This suggests that RhoA activation stimulates PKCζ phosphorylation, a state required for its activation (33, 34). Next, we determined the proliferative and apoptotic effects of PKCζ on TRAMP cells. TRAMP cells were transfected with constitutively active (myristoylated), wild-type, and dominant-negative (K281W) PKCζ plasmids (Fig. 6B), and examined by flow cytometry for cell cycle progression and apoptosis. As can be seen in Fig. 6C, cells expressing dominant-negative PKCζ showed reduced proliferative rates and were protected from apoptotic cell death, similar to T19N cells. On the other hand, expression of constitutively active PKCζ resulted in enhanced proliferation and increased apoptosis, similar to Q63L cells.

The Effects of RhoA on Akt and p70S6 Kinase May Be Mediated by PKCζ. Recent reports in the literature indicate that PKCζ acts upstream of both p70S6 kinase and Akt activation (35–37). We, therefore, determined whether PKCζ influenced the activation of these proteins in TRAMP cells. Akt phosphorylation was inhibited by the expression of myristoylated PKCζ but was enhanced by the expression of dominant negative PKCζ (Fig. 7A). On the other hand, expression of constitutively active PKCζ in TRAMP cells induced enhanced phosphorylation of p70S6 kinase (Fig. 7B), which indicated that PKCζ causes the same changes in phosphorylation of Akt and p70S6.
Fig. 6. In A, immunoblot (top panel) was stained with a phospho-specific PKCζ antibody (Thr410) to demonstrate PKCζ activation. The T19N cells expressed significantly less phospho-PKCζ (top panel). The same blot was stripped and then restained with a total PKCζ antibody to demonstrate equal loading (bottom panel). B, immunoblot stained with phospho-specific PKCζ antibody (top panel). Transfection with constitutively active, myristoylated PKCζ (myr PKCζ) resulted in increased expression of activated PKCζ, whereas expression of the kinase-dead PKCζ mutant (K281W) inhibited PKCζ activation (top panel). Wi-PKCζ, the wild-type PKCζ construct. The same immunoblot was stripped and restained with total PKCζ to demonstrate equal loading (bottom panel). C, cell proliferation and apoptosis observed in TRAMP cells expressing PKCζ mutant proteins as detected by flow cytometry. Cells expressing control or mutant proteins were plated onto 100 mm dishes and incubated for 48 h in the presence of serum. The cells were trypsinized, and one-half were stained with PI for the evaluation of cell proliferation, and the other half were stained with Annexin V for apoptosis (without serum starvation, unlike data presented in Fig. 3A). Expression of K281W reduced cell proliferation and enhanced cell survival. Expression of myr-PKCζ on the other hand, increased cell proliferation and accelerated apoptotic cell death. The data represent the mean of two experiments.

kinase as does RhoA. These data suggest that the effects of RhoA may be mediated by PKCζ.

DISCUSSION

In a previous publication, we showed that RhoA activation stimulates proliferation of murine prostate cancer (TRAMP) cells (3). In this article, we show, in addition, that RhoA induced apoptosis in these cells because inhibition of RhoA caused enhanced cell survival. The molecular mechanisms underlying RhoA-induced cell growth and apoptosis were also investigated. These investigations resulted in several novel observations: (a) inhibition of RhoA caused enhanced phosphorylation of the cell-survival protein Akt (also known as protein kinase B); (b) activation of RhoA stimulated p70S6 kinase overexpression and phosphorylation; and (c) RhoA stimulated the activation of the atypical PKC isoform, PKCζ. Data presented in this paper suggest that the effects of RhoA on TRAMP cell proliferation and apoptosis are mediated by PKCζ.

In mammalian cells, signal transduction leading to cell proliferation and survival may proceed via the MAPK or the PI3K pathways (10). Data presented in this report show that in cells expressing active RhoA (TRAMP and Q63L cells), the PI3K pathway plays a greater role than does the MAPK pathway in cell proliferation and survival. This is indicated by the data showing that the PI3K inhibitor LY 294002, but not the MAPK inhibitor PD 98059, induced growth arrest and apoptosis in these cells. Again, p70S6 kinase, which mediates proliferation downstream of PI3K, was overexpressed in the highly proliferative RhoA-active cell lines (TRAMP and Q63L). Additional data supporting a major role for p70S6 kinase in cell proliferation, in both the presence and the absence of active RhoA, came from the inhibition of cell growth by the immunosuppressant rapamycin, which prevented the activation of p70S6 kinase. These data suggest that in the absence of active RhoA, both the p70S6 kinase- and the MAPK-dependent proliferative pathways are activated in TRAMP cells. However, the expression of active RhoA in these cells causes the enhanced activation of the p70S6 kinase-dependent proliferation pathway, leading to increased proliferative rates.

Another novel observation reported here is that, unlike p70S6 kinase, in the absence of RhoA activation, Akt is phosphorylated (activated). This is accompanied by enhanced cell survival in response to serum deprivation in cells expressing inactive RhoA. This suggests the suppression of Akt activation and cell survival by active RhoA.

This is in contrast to a recent report indicating that dominant-negative RhoA inhibited TGFβ-induced phosphorylation of Akt at Ser473, whereas constitutively active RhoA increased the basal phosphorylation of Akt in NMuMG mammary epithelial cells (38). In support of our observations, however, stable transfectants of NIH3T3 cells, expressing a plasmid containing K-ras mutated at codon 12, exhibited enhanced resistance to apoptosis accompanied by higher AKT/protein kinase B activation and RhoA underexpression (39). This suggests that in certain cells, the presence of active RhoA may be associated with Akt inactivation. Again, earlier studies suggested that p70S6...
kinase activation was mediated by Akt, because p70S6 kinase was stimulated by active mutants of Akt in cotransfection assays (40, 41). However, it now appears that Akt mediates p70S6 kinase activation only as a function of constitutive membrane localization (17). Our data also suggest that p70S6 kinase and Akt are on parallel pathways downstream of PI3K because in cells expressing active RhoA, p70S6 kinase was activated and Akt was inactivated, whereas the opposite was true in cells expressing inactive RhoA.

To determine a cause for this difference in protein activation in the presence or absence of active RhoA, we examined PKCζ, which was previously shown to stimulate p70S6 kinase activity (37) but down-regulate Akt (35, 36). PKCζ, PKB/Akt, and p70S6 kinase are all substrates for PDK1. Whereas both p70S6 kinase and Akt can be phosphorylated by PDK1, p70S6 kinase can also be phosphorylated by PKCζ by direct binding (37), although Akt can be dephosphorylated by the same PKC isoform by direct binding as well (35). In our cells, PKCζ was stimulated in the presence of active RhoA and inactivated in its absence. Similar to RhoA, PKCζ activation also stimulated TRAMP cell proliferation, whereas inhibition of its activity inhibited TRAMP cell growth. Similarly, the activation of PKCζ stimulated apoptosis, whereas the inhibition of its activity stimulated cell survival (inhibited apoptosis). Again, like RhoA, inactivation of PKCζ activated Akt, although less active Akt was observed in cells expressing active PKCζ. Thus, our data suggest that PKCζ mediates the effects of RhoA on cell proliferation and apoptosis, and especially the inactivation of Akt in RhoA-active cells.

Because p70S6 kinase, one downstream effector of PI3K, is activated in cells expressing active RhoA, whereas Akt, another downstream effector of PI3K, is activated in cells expressing inactive RhoA, it is likely that PI3K is activated both in the presence and the absence of active RhoA. Therefore, RhoA probably affects PKCζ directly. In fact, it has been shown that pretreatment with C3 transferase, a specific inhibitor of Rho small GTPases (RhoA, RhoB, RhoC), blocks membrane translocation of PKCζ but not that of PKCα (42). Plasma membrane translocation is required for the activation of all PKC isoforms (42). In addition, it was recently shown in a cell-free system that the level of PKCζ activity was potentiated 5-fold over basal levels by the addition of RhoA, whereas the level of PKCα resulted in only a slight increase (see Fig. 1A in Ref. 43). Thus, it is likely that RhoA activates PKCζ directly, and that PKCζ in turn activates p70S6 kinase and inactivates Akt. This effect would be additive to that of PDK1 on Akt and p70S6 kinase. This is suggested by the evidence that the inhibition of PI3K with LY 294002 inhibits p70S6 kinase and cell proliferation, as well as Akt and cell survival.

A scheme depicting the possible signaling pathway is depicted in Fig. 8.

In conclusion, we have shown that RhoA-active cells exhibit high rates of cell proliferation and low rates of cell survival, whereas RhoA-inactive cells exhibit high survival rates. The significance of these observations is that in the presence of active RhoA, the rate of proliferation exceeds the rate of cell death, and the cells exhibit a proliferative phenotype. On the other hand, in the absence of active RhoA, the apoptosis rate is low, and the cells exhibit a cell survival phenotype. Growth of prostate cancer is determined by the relative rates of cell proliferation and cell death (apoptosis or programmed cell death). The transition of late-stage high-grade intraepithelial prostatic neoplasia (PIN) into growing localized and metastatic prostatic cancer is primarily caused by a decreased apoptotic death rate (44). From our present studies, it appears that the state of RhoA activation may determine whether prostate tumor cells display a proliferative or cell survival phenotype.

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