[CANCER RESEARCH 59, 1449–1453, April 1, 1999]

Advances in Brief

Antiapoptotic Signaling in LNCaP Prostate Cancer Cells: A Survival Signaling Pathway Independent of Phosphatidylinositol 3′-Kinase and Akt/Protein Kinase B[1]

Jonathan P. Carson, George Kulik, and Michael J. Weber[2]

Department of Microbiology and Cancer Center, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908

Abstract

Constitutive activation of the phosphatidylinositol 3′-kinase (PI3 kinase)-Akt/protein kinase B (PKB) "survival signaling" pathway is a likely mechanism by which many cancers become refractory to cytotoxic therapy. In LNCaP prostate cancer cells, the PTEN phosphoinositide phosphatase is inactivated, leading to constitutive activation of Akt/PKB and resistance to apoptosis. However, apoptosis and inactivation of Akt/PKB can be induced in these cells by treatment with PI3 kinase inhibitors. Surprisingly, androgen, epidermal growth factor, or serum can protect these cells from apoptosis, even in the presence of PI3 kinase inhibitors and without activation of Akt/PKB, indicating the activity of a novel, Akt/PKB-independent survival pathway. This pathway blocks apoptosis at a level prior to caspase 3 activation and release of cytochrome *c* from mitochondria.

Introduction

The sensitivity of tumor cells to apoptosis determines their ability to escape cytotoxic therapies (1–3) and to survive under adverse physiological circumstances, such as reduced oxygen (4–6) and sites of metastasis (7). Recently, a survival-regulating signal transduction pathway has been identified that is important in determining sensitivity to apoptosis in neural (8–10), fibroblastic (11–13), epithelial (11, 14), and hematopoietic cells (15). This pathway, which is engaged by several "survival factors" such as IGF[3]-I and interleukin 3, involves, sequentially, the activation of PI3 kinase, the production of 3′-phosphoinositides, activation and recruitment of phospholipid-dependent kinases, and their phosphorylation of the protein kinase Akt/PKB on serine 473 and threonine 308 (11, 16–19). Phosphorylation on these sites activates Akt/PKB, which then inhibits apoptosis by phosphorylation and inactivation of the proapoptotic proteins BAD (20, 21), Caspase 9 (22), and perhaps other proteins as well.

The PTEN phosphatase has been shown to be capable of dephosphorylating phosphoinositides. Cells in which this phosphatase activity is lost display constitutively elevated steady-state levels of phosphatidylinositol (3′,4′,5′) phosphate (23) and constitutive activation of the Akt/PKB survival pathway. Tumor cells affected in this manner may escape dependence on extracellular survival factors such as IGF-I, which induce survival signals through PI3 kinase to activate Akt/PKB. Such cells are expected to gain resistance to agents that induce apoptosis, and this could provide the selective pressure that accounts for the fact that PTEN is inactivated in ~50% of prostate tumors (24–27).

When prostate cancer first presents, the disease is usually responsive to androgen ablation therapy, which induces the tumors to regress. This indicates that androgen functions as a survival factor for at least some prostate cell types (28, 29). However, the disease generally recurs within 1–3 years of treatment, and the recurrent tumors no longer require androgen for growth or for survival (30). In addition, these tumors tend to be highly resistant to conventional cytotoxic agents such as cisplatin (31). The observation that *PTEN* is frequently disrupted in late-stage prostate cancers underscores the importance of deregulated survival signaling in tumor progression.

The most widely used *in vitro* model of prostate cancer is the LNCaP cell line (32). LNCaP contains a frameshift mutation in the *PTEN* gene (26). Consistent with the defective PTEN, we report here that Akt/PKB is constitutively phosphorylated in LNCaP, even after 24 h of culture in serum-free media. As expected, growth factor withdrawal by itself does not induce apoptosis in these cells. However, treatment of serum-starved LNCaP with inhibitors of PI3 kinase diminishes the phosphorylation of Akt/PKB and results in induction of apoptosis. This finding is in agreement with the concept that it is the constitutive activation of the PI3 kinase/Akt pathway that renders these cells capable of surviving without exogenous growth and survival factors. However, various exogenous growth and survival factors, including EGF, androgen, and serum, were found to be capable of inhibiting apoptosis, even in the presence of PI3 kinase inhibitors. These results provide evidence for the existence of a novel survival pathway in prostate cancer cells that is independent of PI3 kinase and Akt/PKB.

Materials and Methods

Antibodies and Other Reagents. Antibodies were from the following sources: anti-Akt, anti-mitogen-activated protein kinase, and anti-phospho-Akt from New England Biolabs (Beverly MA); anti-PARP from Upstate Biotechnology (Lake Placid, NY); anti-cytochrome *c* from PharMingen (San Diego, CA). Chemicals and reagents were from Sigma Chemical Co. unless otherwise specified. Tissue culture and reagents were from Life Technologies, Inc. (Gaithersburg, MD), and IGF-I was a gift from Thomas Sturgill (University of Virginia). Recombinant EGF was from Upstate Biotechnology, and R1881 was from NEN Life Science Products (Boston, MA).

Cell Culture. LNCaP cells were a gift from the laboratory of Dr. Leland Chung (University of Virginia) and were passaged in RPMI 1640 with 10% FCS in 6% CO_2 at 37°C. DU145 cells were obtained from American Type Culture Collection and were maintained according to the guidelines recommended by the agency.

Stimulation of Cells with Growth Factors. In Fig. 1, we stimulated semiconfluent, 10-cm cell culture dishes of DU145 and LNCaP cells according to the following time scheme. Cells were first washed twice with 8 ml of serum-free RPMI 1640 and replaced with serum-free RPMI 1640. R1881 (5 nM) was added at this time. Twenty-four h later, LY294002 was added (20 μM); at which time the medium was changed in all dishes with fresh serum-

Received 1/13/99; accepted 2/15/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by NIH Grants CA39076, GM47332, and CA76500 and a gift from CaPCURE.

[2] To whom requests for reprints should be addressed, at Dept. of Microbiology, University of Virginia School of Medicine, Box 441, Charlottesville, VA 22908. Phone: (804) 924-5022; Fax: (804) 982-0689; E-mail: MJW@Virginia.edu.

[3] The abbreviations used are: IGF, insulin-like growth factor; PKB, protein kinase B; EGF, epidermal growth factor; PI3 kinase, phosphatidylinositol 3′-kinase; PARP, poly-(ADP-ribose) polymerase; DAPI, 4′,6-diamidino-2-phenylindole.

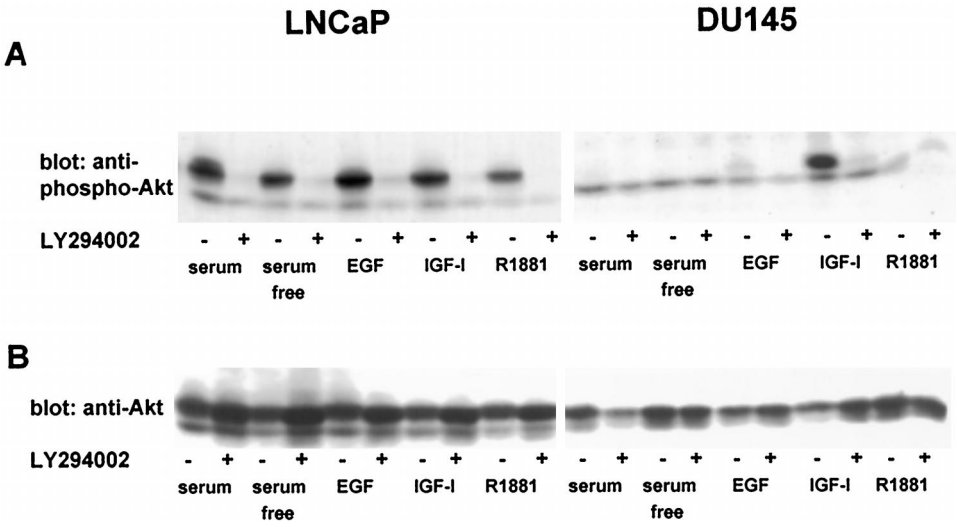

Fig. 1. Inhibition of constitutive Akt/PKB phosphorylation in LNCaP and stimulated Akt/PKB phosphorylation in DU145. *A*, phosphorylation of Akt/PKB in the presence and absence of LY294002 and survival factors as depicted by anti-phospho-Akt/PKB Western blot that reflects the active form of Akt/PKB. *B*, loading control of Akt/PKB, shown here as a Western blot using anti-Akt/PKB antibody.

free RPMI 1640. Forty-five min after addition of LY294002, EGF (100 ng/ml) and IGF-I (500 μg/ml) were added. EGF and IGF-I remained in the media for 15 min before all cells were harvested on ice for Western blot analysis.

Apoptosis. Cells were maintained on 15-cm dishes grown to ~75% confluence and split to an equal density onto 10-cm dishes, each of which contained a sterile glass coverslip. Twenty-four to 48 h later, the medium on the dishes was washed twice with 8 ml of serum-free RPMI and replaced with serum free RPMI for 24 h. LY294002 (20 μM) was added after 24 h of serum-free conditions, unless otherwise specified, after a change of media in all dishes. EGF was added at a concentration of 100 ng/ml simultaneously with LY294002. Whenever R1881 was administered (Figs. 1–4), it was added (5 nm) during the 24-h preincubation with serum-free media as well as during the time of subsequent treatment with LY294002. After treatment with LY294002, cells were harvested on ice. Coverslips were removed from the dishes and fixed in 3% formalin in PBS. Both adherent and floating cells remaining in the dish were collected and equally divided into separate samples for the Cell Death Detection ELISA (Boehringer Mannheim, Indianapolis, IN) and for caspase assays. Cells were then pelleted at 200 $\times$ *g* for 5 min. Samples to be assayed by the Cell Death ELISA were prepared as directed by the instructions in the kit. Samples to be used for caspase 3 activity assays were rinsed once in cold PBS, repelleted, and treated as instructed by the manufacturer of the Ac-DEVD-AMC substrate (United Biomedical, Inc., Lake Success, NY or Bio-Rad, Hercules, CA).

Formalin-fixed coverslips were then stained with DAPI, rinsed in distilled H_2O, and mounted onto slides using Vectashield (Vector Laboratories, Burlingame, CA) for analysis under the microscope. Each slide was counted in a blind fashion for the number of apoptotic and nonapoptotic nuclei in randomly selected visual fields under $\times$60 magnification. At least 2500 nuclei were counted per data point. All data points depicted in the graphs of Figs. 2–4 represent the average of three separate dishes from the same experiment, where the error bar represents 1 SD.

Hypotonic Lysis for Anti-Cytochrome *c* Western Blots. The following protocol is an adaptation of the methods to obtain a cytosol-specific, mitochondria-free cell lysate, originally described by Bossy-Wetzel *et al.* (33). LNCaP cells were grown to ~75% confluence on 15-cm tissue culture dishes. Cells were then washed twice with serum-free RPMI 1640 (Life Technologies) and maintained in serum-free conditions for 24 h. After this preincubation, cells were induced to undergo apoptosis with 20 μM LY294002 in the presence or absence of R1881, EGF (100 ng/ml), or LY294002. A separate dish of cells kept solely in serum-free conditions was used as a negative control for apoptosis. As in previous experiments, certain samples were treated with R1881 (5 nM) during a 24-h period of incubation prior to administration of LY294002. After 12 h of incubation, both media supernatants and adherent cells from each sample were collected and centrifuged for 5 min at 200 $\times$ *g*. After aspirating supernatants, each pellet was then placed on ice and lysed in 300-μl hypotonic solution [220 mM mannitol, 68 mM sucrose, 50 mM PIPES-KOH (pH 7.4), 50 mM KCl, 5 mM EDTA, 2 mM $MgCl_2$, 1 mM DTT, protease inhibitors] by trituration 10 times. After an incubation of 45 min on ice, the resuspended pellets were again centrifuged at 200 $\times$ *g* for 10 min. Supernatants were collected and transferred to 1.5-ml microcentrifuge tubes (Eppendorf Scientific USA, Westbury, NY), where they were centrifuged at 14,000 rpm for 10 min. The supernatants were transferred to new microcentrifuge tubes and stored until use for SDS-PAGE.

Fifty μg of protein from each sample of hypotonic cell lysis solution were loaded per lane and separated by 8–18% PAGE. Protein was transferred to nitrocellulose membranes, which were used in Western blots in which the primary antibody was directed against cytochrome *c*.

Results

Inhibition of Constitutive Akt/PKB Phosphorylation by LY294002 in LNCaP. When LNCaP cells were placed in serum-free medium for 24 h, Akt/PKB remained in its active, phosphorylated state in the absence of any external stimulation (Fig. 1). In contrast, in the androgen-unresponsive prostate cancer cell line, DU145, Akt/PKB was not phosphorylated unless the cells were stimulated with IGF-I. Elevated phosphorylation of Akt/PKB in LNCaP was inhibited by treating cells with the PI3 kinase inhibitor LY294002 for 1 h, indicating that constitutive phosphorylation of Akt/PKB on the regulatory residue S473 was dependent on constitutive production of 3′-phosphoinositides. Inhibition of PI3 kinase activity was sufficient to inhibit phosphorylation of Akt/PKB in LNCaP and DU145 cells stimulated with EGF and IGF-I (Fig. 1). These effects were seen both with LY294002 and another PI3 kinase inhibitor, wortmannin (data not shown). Thus, constitutive Akt/PKB phosphorylation in LNCaP and induced levels of Akt/PKB phosphorylation in DU145 are dependent on production of 3′-phosphoinositides.

LY294002 Causes Apoptosis in LNCaP Cells in Serum-free Medium. Because LY294002 blocks a crucial pathway involved in cell survival, we tested whether the compound was sufficient to induce apoptosis in LNCaP cells. After 24 h of culture in serum-free medium, LNCaP cells were treated with LY294002 for 0, 3, 6, 12, and 24 h. For comparison, cells maintained under normal growth conditions (10% FCS, RPMI), as well as untreated cells maintained under serum-free conditions, were collected at corresponding time points. By analyzing nuclear morphology (Fig. 2*A*), caspase 3 activity (Fig. 2*B*), and DNA fragmentation (Fig. 2*C*), we found that 20 μM LY294002 caused increased apoptosis as early as 3 h after addition to the culture media.

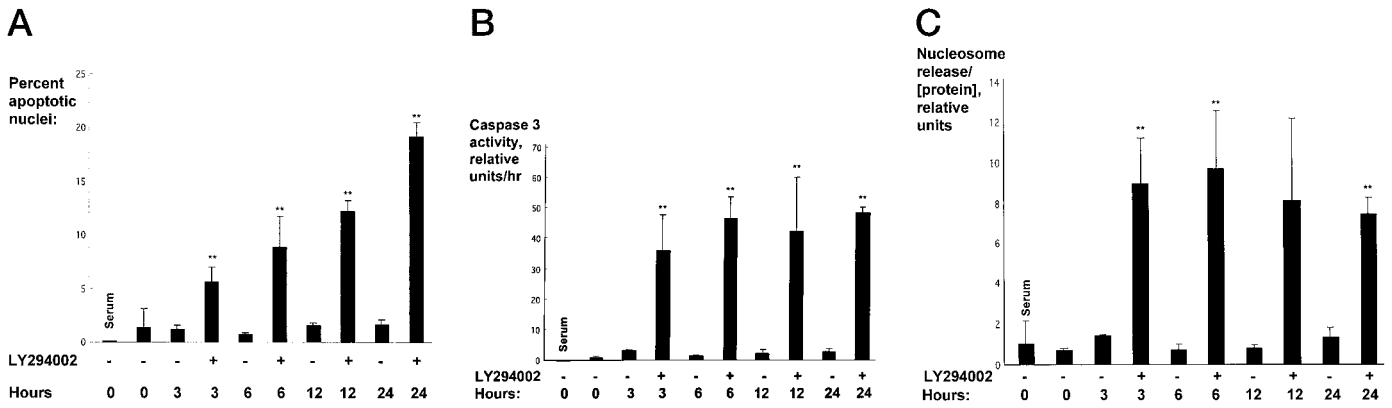

Fig. 2. Time course of LY294002-mediated apoptosis in LNCaP in serum-free media. *A*, percentage of condensed and fragmented nuclei as visualized by DAPI fluorescence microscopy. Bars, SD. *B*, caspase 3 activity in relative rate of cleavage of fluorometric, Ac-DEVD-AMC substrate. *Bars*, SD. *C*, readout of Cell Death Detection ELISA, proportional to concentration of cleaved nucleosome fragments and normalized to protein concentration. *Bars*, SD. **, significance of $P < 0.05$.

Serum, EGF, and R1881 Prevent LY294002-mediated Apoptosis. To determine whether putative growth and survival factors such as EGF, R1881, and serum can inhibit apoptosis under conditions where Akt/PKB is inactive, we asked whether the ability of LY294002 to induce apoptosis could be blocked by these agonists. If cells were maintained under serum-free conditions for 24 h, addition of LY294002 triggered a vigorous induction of apoptosis within 12 h, as depicted by DAPI-stained nuclear morphology (Fig. 3*A*) and the Cell Death Detection ELISA (Fig. 3*B*). The chronic presence of 10% serum inhibited this cell death. EGF (100 ng/ml) was sufficient to block LY294002-mediated apoptosis when coadministered for 12 h with LY294002. When 5 nM R1881 was added as a pretreatment during the 24 h of prior serum-free culture, a diminution of LY294002-mediated apoptosis was likewise observed. Because LY294002 was still able to inhibit the phosphorylation of Akt/PKB regardless of whether EGF, R1881, or serum were present at both early (Fig. 1) and late (data not shown) time points, we conclude that these agonists can stimulate a novel survival pathway in LNCaP that is independent of Akt/PKB as well as PI3 kinase.

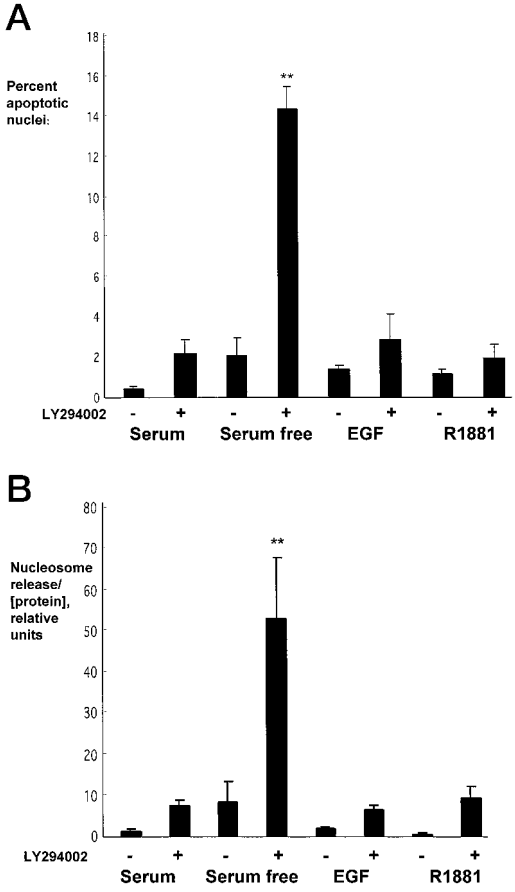

Fig. 3. Protection from LY294002-mediated apoptosis by 10% FCS, EGF, and R1881 in LNCaP cells. *A*, percentage of condensed and fragmented nuclei as visualized by DAPI fluorescence microscopy. *Bars*, SD. *B*, Cell Death Detection ELISA readout. **, significance of $P < 0.05$. *Bars*, SD.

EGF and R1881 Prevent LY294002-mediated Caspase 3 Activation and Cytochrome *c* Release. In current models of apoptosis, an initial proapoptotic stress may induce the release of cytochrome *c* from mitochondria into the cytosol. Cytochrome *c* interacts with Apaf-1 and facilitates the oligomerization of pro-caspase 9 immediately prior to activation of the effector caspase, caspase 3 (34, 35). As a step in elucidating the mechanism by which the novel survival signaling pathway operates, we determined whether this pathway interferes with activation or activity of effector caspases or of cytochrome release. Fig. 4*A* shows that LY294002 is able to induce caspase 3 activation in LNCaP cells under serum-free conditions and that this activation is almost entirely blocked by EGF or by prior treatment with androgen.

To determine whether EGF or R1881 protect LNCaP cells from LY294002-mediated apoptosis by interfering with cytochrome *c* release, a nondenaturing, hypotonic lysis was used to collect cytosolic lysates of LNCaP following treatments, and the presence of cytochrome *c* was assessed by Western blotting. LY294002 induced an increase in the amount of nonmitochondrial, cytosolic cytochrome *c* after 12 h (Fig. 4). This release correlates with the cleavage of PARP by caspases (data not shown). EGF (Fig. 4*A*) and R1881 (Fig. 4*B*), however, inhibit both PARP cleavage (data not shown) and cytochrome *c* release. Therefore, we conclude that EGF- and R1881-mediated survival occurs upstream of mitochondrial cytochrome *c* release and caspase 3 activation.

Discussion

In this work, we report that Akt/PKB is constitutively phosphorylated in LNCaP cells and that pharmacological inhibition of PI3 kinase and Akt/PKB induces apoptosis when other growth and survival factors are not present. This is consistent with the PI3 kinase-Akt/PKB survival signaling pathway being constitutively operative in these cells. Surprisingly, apoptosis triggered by blockade of PI3 kinase could be prevented by serum, androgen, or EGF, indicating the

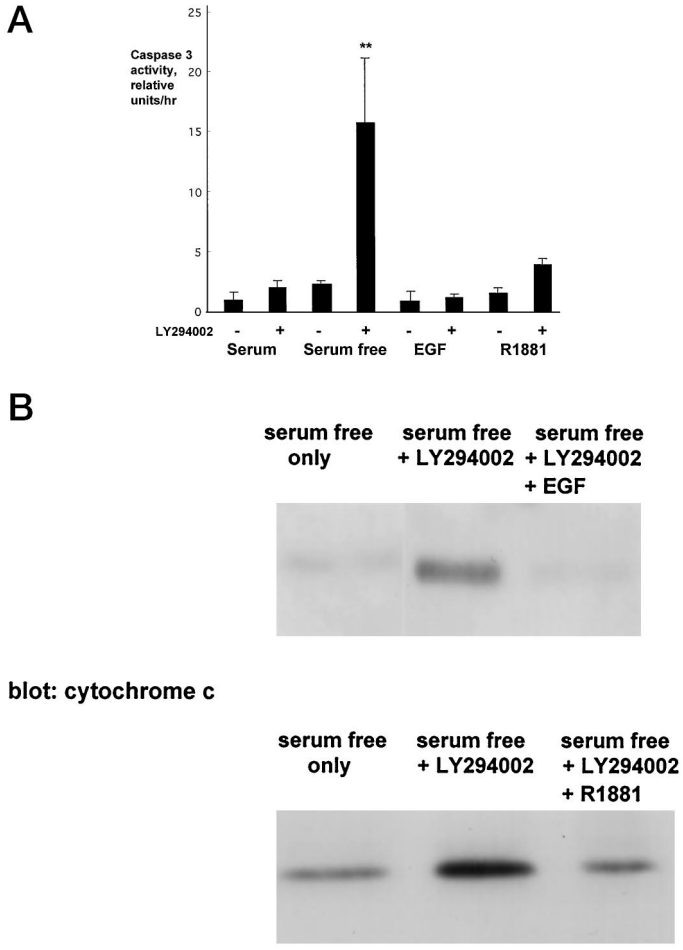

Fig. 4. Inhibition of caspase 3 activity and cytochrome *c* release by EGF and R1881 in LNCaP cells. *A*, caspase 3 activity after treatment with LY294002 in the presence or absence of protective factors. **, significance of $P < 0.05$. *Bars*, SD. *B*, cytochrome *c* release from mitochondria as demonstrated by representative Western blot of cytosol from LNCaP with cytochrome *c*-specific antibodies.

presence of a novel survival signaling pathway independent of PI3 kinase and Akt/PKB. We showed recently that such a pathway could function in cells engineered to overexpress the IGF-I receptor (36), but this is the first report of such a survival signaling pathway in human tumor cells.

The finding that Akt/PKB is constitutively phosphorylated in LNCaP cells is in accordance with the observation that PTEN is not expressed in LNCaP due to a frameshift mutation (26). In other systems where *PTEN* is deleted, the phosphatase is no longer able to regulate the accumulation of phosphatidylinositol (3′,4′,5′) phosphates, resulting in constitutive elevation of Akt/PKB activity (23).

Because cytotoxic cancer therapy operates at least in part by inducing apoptosis, treatment of tumors likely generates considerable selective pressure for the expansion of cells that are resistant to apoptosis. The frequent inactivation of PTEN and consequent activation of the PI3 kinase-Akt/PKB survival signaling pathway may reflect a response to these selective pressures. These considerations have raised the possibility that inhibition of the PI3 kinase-Akt/PKB survival pathway might sensitize cancer cells to chemotherapy or radiation. However, the existence in tumor cells of a survival signaling pathway that does not depend on PI3 kinase and Akt/PKB indicates that inhibition of Akt/PKB alone may not be sufficient to sensitize tumors to therapy-induced apoptosis. Consistent with this suggestion is the report that the PI3 kinase inhibitor, wortmannin, fails to suppress the growth of implanted xenograft tumors in nude mice (37).

Little is known about the mechanism by which the Akt/PKB-independent pathway functions. We found that caspase 3 activation and cytochrome *c* release from mitochondria were inhibited by this pathway, properties shared by antiapoptotic members of the Bcl2 family (38). We do not know whether the Akt/PKB-independent pathway makes use of Bcl2 or members of the Bcl2 family. Current research is aimed at understanding the molecular nature of this pathway so that it can be subjected to experimental and therapeutic manipulation.

References

1. Haq, R., and Zanke, B. Inhibition of apoptotic signaling pathways in cancer cells as a mechanism of chemotherapy resistance. Cancer Metastasis Rev., *2:* 233–239, 1998.
2. Fisher, D. E. Apoptosis in cancer therapy: crossing the threshold. Cell, *78:* 539–542, 1994.
3. Lowe, S. W., Bodis, S., McClatchey, A., Remington, L., Ruley, H. E., Fisher, D. E., Housman, D. E., and Jacks, T. p53 status and the efficacy of cancer therapy *in vivo*. Science (Washington DC), *266:* 807–810, 1994.
4. Graeber, T. G., Osmanian, C., Jacks, T., Housman, D. E., Koch, C. J., Lowe, S. W., and Giaccia, A. J. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. Nature (Lond.), *379:* 88–91, 1996.
5. Schmaltz, C., Hardenbergh, P. H., Wells, A., and Fisher, D. E. Regulation of proliferation-survival decisions during tumor cell hypoxia. Mol. Cell. Biol., *18:* 2845–2854, 1998.
6. Magnusson, K. P., Satalino, R., Qian, W., Klein, G., and Wiman, K. G. Is conversion of solid into more anoxic ascites tumors associated with p53 inactivation? Oncogene, *17:* 2333–2337, 1998.
7. McConkey, D. J., Greene, G., and Pettaway, C. A. Apoptosis resistance increases with metastatic potential in cells of the human LNCaP prostate carcinoma line. Cancer Res., *56:* 5594–5599, 1996.
8. Dudek, H., Datta, S. R., Franke, T. F., Birnbaum, M. J., Yao, R., Cooper, G. M., Segal, R. A., Kaplan, D. R., and Greenberg, M. E. Regulation of neuronal survival by the serine-threonine protein kinase Akt. Science (Washington DC), *275:* 661–665, 1997.
9. Bertollini, L., Ciotti, M. T., Cherubini, E., and Cattaneo, A. Neurotrophin-3 promotes the survival of oligodendrocyte precursors in embryonic hippocampal cultures under chemically defined conditions. Brain Res., *746:* 19–24, 1997.
10. Franke, T. F., Kaplan, D. R., and Cantley, L. C. PI3K: downstream AKTion blocks apoptosis. Cell, *88:* 435–437, 1997.
11. Kulik, G., Klippel, A., and Weber, M. J. Antiapoptotic signalling by the insulin-like growth factor I receptor, phosphatidylinositol 3-kinase, and Akt. Mol. Cell Biol., *17:* 1595–1606, 1997.
12. Harrington, E. A., Bennett, M. R., Fanidi, A., and Evan, G. I. c-Myc-induced apoptosis in fibroblasts is inhibited by specific cytokines. EMBO J., *13:* 3286–3295, 1994.
13. Jung, Y., Miura, M., and Yuan, J. Suppression of interleukin-1β-converting enzyme-mediated cell death by insulin-like growth factor. J. Biol. Chem., *271:* 5112–5117, 1996.
14. Khwaja, A., Rodriguez-Viciana, P., Wennstrom, S., Warne, P. H., and Downward, J. Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. EMBO J., *16:* 2783–2793, 1997.
15. Muta, K., and Krantz, S. B. Apoptosis of human erythroid colony-forming cells is decreased by stem cell factor and insulin-like growth factor I as well as erythropoietin. J. Cell Physiol., *156:* 264–271, 1993.
16. Alessi, D. R., and Cohen, P. Mechanism of activation and function of protein kinase B. Curr. Opin. Genet. Dev., *8:* 55–62, 1998.
17. Kohn, A. D., Takeuchi, F., and Roth, R. A. Akt, a pleckstrin homology domain containing kinase, is activated primarily by phosphorylation. J. Biol. Chem., *271:* 21920–21926, 1996.
18. Alessi, D. R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P., and Hemmings, B. A. Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J., *15:* 6541–6551, 1996.
19. Stokoe, D., Stephens, L. R., Copeland, T., Gaffney, P. R., Reese, C. B., Painter, G. F., Holmes, A. B., McCormick, F., and Hawkins, P. T. Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B. Science (Washington DC), *277:* 567–570, 1997.
20. del Peso, L., Gonzalez-Garcia, M., Page, C., Herrera, R., and Nunez, G. Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science (Washington DC), *278:* 687–689, 1997.
21. Datta, S. R., Dudek, H., Tao, X., Masters, S., Fu, H., Gotoh, Y., and Greenberg, M. E. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell, *91:* 231–241, 1997.
22. Cardone, M. H., Roy, N., Stennicke, H. R., Salvesen, G. S., Franke, T. F., Stanbridge, E., Frisch, S., and Reed, J. C. Regulation of cell death protease caspase-9 by phosphorylation. Science (Washington DC), *282:* 1318–1321, 1998.
23. Stambolic, V., Suzuki, A., de la Pompa, J. L., Brothers, G. M., Mirtsos, C., Sasaki, T., Ruland, J., Penninger, J. M., Siderovski, D. P., and Mak, T. W. Negative

regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell, *95:* 29–39, 1998.
24. Pesche, S., Lati, A., Muzeau, F., Cussenot, O., Fournier, G., Longy, M., Eng, C., and Lidereau, R. PTEN/MMAC1/TEP1 involvement in primary prostate cancers. Oncogene, *16:* 2879–2883, 1998.
25. Whang, Y. E., Wu, X., Suzuki, H., Reiter, R. E., Tran, C., Vessella, R. L., Said, J. W., Isaacs, W. B., and Sawyers, C. L. Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. Proc. Natl. Acad. Sci. USA, *95:* 5246–5250, 1998.
26. Vlietstra, R. J., van Alewijk, D. C., Hermans, K. G., van Steenbrugge, G. J., and Trapman, J. Frequent inactivation of PTEN in prostate cancer cell lines and xenografts. Cancer Res., *58:* 2720–2723, 1998.
27. Cairns, P., Okami, K., Halachmi, S., Halachmi, N., Esteller, M., Herman, J. G., Jen, J., Isaacs, W. B., Bova, G. S., and Sidransky, D. Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. Cancer Res., *5:* 4997–5000, 1997.
28. Gao, J., and Isaacs, J. T. Development of an androgen receptor-null model for identifying the initiation site for androgen stimulation of proliferation and suppression of programmed (apoptotic) death of PC-82 human prostate cancer cells. Cancer Res., *58:* 3299–3306, 1998.
29. Banerjee, P. P., Banerjee, S., Tilly, K. I., Tilly, J. L., Brown, T. R., and Zirkin, B. R. Lobe-specific apoptotic cell death in rat prostate after androgen ablation by castration. Endocrinology, *136:* 4368–4376, 1995.
30. Tang, D. G., and Porter, A. T. Target to apoptosis: a hopeful weapon for prostate cancer. Prostate, *32:* 284–293, 1997.
31. Korman, L. B. Treatment of prostate cancer. Clin. Pharm., *8:* 412–424, 1989.
32. Horoszewicz, J. S., Leong, S. S., Chu, T. M., Wajsman, Z. L., Friedman, M., Papsidero, L., Kim, U., Chai, L. S., Kakati, S., Arya, S. K., and Sandberg, A. A. The LNCaP cell line–a new model for studies on human prostatic carcinoma. Prog. Clin. Biol. Res., *37:* 115–132, 1980.
33. Bossy-Wetzel, E., Newmeyer, D. D., and Green, D. R. Mitochondrial cytochrome *c* release in apoptosis occurs upstream of DEVD-specific caspase activation, and independently of mitochondrial transmembrane depolarization. EMBO J., *17:* 37–49, 1998.
34. Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S. M., Ahmad, M., Alnemri, E. S., and Wang, X. Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell, *91:* 479–489, 1997.
35. Hakem, R., Hakem, A., Duncan, G. S., Henderson, J. T., Woo, M., Soengas, M. S., Elia, A., de la Pompa, J. L., Kagi, D., Khoo, W., Potter, J., Yoshida, R., Kaufman, S. A., Lowe, S. W., Penninger, J. M., and Mak, T. W. Differential requirement for caspase 9 in apoptotic pathways in vivo. Cell, *94:* 339–352, 1998.
36. Kulik, G., and Weber, M. J. Akt-dependent and -independent survival signaling pathways utilized by insulin-like growth factor I. Mol. Cell. Biol. *18:* 6711–6718, 1998.
37. Schultz, R. M., Merriman, R. L., Andis, S. L., Bonjouklian, R., Grindey, G. B., Rutherford, P. G., Gallegos, A., Massey, K., and Powis, G. *In vitro* and *in vivo* antitumor activity of the phosphatidylinositol-3-kinase inhibitor, wortmannin. Anticancer Res., *15:* 1135–1139, 1995.
38. Green, D. R., and Reed, J. C. Mitochondria and apoptosis. Science (Washington DC), *281:* 1309–1312, 1998.

Antiapoptotic Signaling in LNCaP Prostate Cancer Cells: A Survival Signaling Pathway Independent of Phosphatidylinositol 3′-Kinase and Akt/Protein Kinase B

Jonathan P. Carson, George Kulik and Michael J. Weber

Cancer Res 1999;59:1449-1453.