[CANCER RESEARCH 59, 2551–2556, June 1, 1999]

Advances in Brief

Regulation of Akt/PKB Activity, Cellular Growth, and Apoptosis in Prostate Carcinoma Cells by MMAC/PTEN[1]

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Abstract

Understanding the functional roles of the molecular alterations that are involved in the oncogenesis of prostate cancer, the second most frequent cause of cancer-related deaths among men in the United States is the focus of numerous investigations. To examine the possible significance of alterations associated with the tumor suppressor gene, *MMAC/PTEN*, in prostate carcinoma, the biological and biochemical effects of MMAC/PTEN expression were examined in LNCaP cells, which are devoid of a functional gene product. Acute expression of MMAC/PTEN via an adenoviral construct resulted in a dose-dependent and specific inhibition of Akt/PKB activation, consistent with the phosphatidylinositol phosphatase activity of MMAC/PTEN. MMAC/PTEN expression induced apoptosis in LNCaP cells, although to a lesser extent than that observed with p53 via an adenoviral construct. However, MMAC/PTEN expression produced a growth inhibition that was significantly greater than that achieved with p53. Overexpression of Bcl-2 in LNCaP cells blocked MMAC/PTEN- and p53-induced apoptosis but not the growth-suppressive effects of MMAC/PTEN, suggesting that the growth regulatory effects of MMAC/PTEN involve multiple pathways. These studies further implicate the loss of *MMAC/PTEN* as a significant event in prostate cancer and suggest that reintroduction of *MMAC/PTEN* into deficient prostate cancer cells may have therapeutic implications.

Introduction

Prostate cancer is the second leading cause of cancer-related deaths among men in the United States, with over 40,000 patients succumbing to the disease in 1997 (1). To further define the molecular basis of oncogenesis for this disease, a number of chromosomal alterations have been identified to frequently occur, including structural changes to 7q, 8p, 10, 13q, and 16q (2, 3). In particular, allelic deletions associated with chromosome 10q, specifically the q23–25 region, have been observed to preferentially occur in the advanced stages of disease (4, 5). In support of the implication that losses associated with 10q are predominantly involved in prostate cancer progression, a previous study has demonstrated that reintroduction of the 10q region into rat prostate tumor cells significantly inhibited their metastatic capabilities but failed to alter their tumorigenicity (6). The combination of these results suggest that molecular alterations to a gene or genes on 10q may be involved in the malignant nature of prostate carcinoma similar to that observed in other cancers (7).

One candidate tumor suppressor gene on 10q, *MMAC/PTEN*, initially identified due to homozygous deletions in gliomas and breast cancer cell lines, was shown to be altered in a number of cancers including prostate carcinoma cells (8, 9). Furthermore, interest in *MMAC/PTEN* was heightened due to the presence of a structural motif for a dual specificity protein phosphatase in the protein product that strongly suggested its involvement in the regulation of signal transduction. Recently, MMAC/PTEN has been shown to have phosphatidylinositol phosphatase activity, specifically for the 3′ position (10), implying its involvement in PI3′K[3]-mediated pathways. More recently, the lipid phosphatase activity has been shown to be essential for its ability to inhibit tumorigenesis and growth inhibition (11, 12). In this regard, our own studies and those of others have shown that reintroduction of *MMAC/PTEN* into cells deficient of a functional gene product modulates the activity of Akt/PKB (13, 14). Akt/PKB, a serine-threonine kinase, has been shown to be involved in a number of proliferative, metabolic, and antiapoptotic pathways that are dependent upon PI3′K signaling to be activated (15).

To examine the effects of MMAC/PTEN expression, an adenovirus was developed to express *MMAC/PTEN* under the control of a cytomegalovirus promoter (14). This adenovirus, Ad-MMAC, was used to assess the biological and biochemical response of prostate carcinomas cells to functional MMAC/PTEN expression. The LNCaP cells were originally derived from a metastatic prostate cancer specimen and represent a model for androgen-sensitive prostate cancer. Furthermore, the effects of MMAC/PTEN expression on LNCaP cells engineered to overexpress Bcl-2, a negative regulator of apoptosis that has been implicated in prostate cancer progression (16), was also examined. In conjunction, the biological effects of MMAC/PTEN expression were compared to the effects of p53 expression via adenoviral vectors in the same cells.

Materials and Methods

Cell Lines and Viral Infections. LNCaP cells were maintained in culture in media supplemented with 10% FCS, as described previously (14). The LNCaP-Bcl-2 cells were developed from LNCaP cells transfected with Bcl-2 on a long terminal repeat promoter, the characterization of which is described elsewhere (17). A recombinant adenovirus containing wild-type MMAC1/PTEN (Ad-MMAC) described previously was used in these studies (14). An adenovirus expressing the enhanced green fluorescent protein (Ad-GFP) was derived from the same vector as Ad-MMAC; the replication-deficient virus without a transgene (Ad-DE1/Ad5-δE1) has been described previously (18), along with the virus expressing the *p53* transgene (19). Viruses were amplified in 293 cells, isolated by cesium chloride gradient or purified as reported previously (14), and titer was determined by absorbance.

Protein Analysis. Subconfluent monolayers of cells were infected with Ad-MMAC, Ad-DE1 at the indicated MOI, or mock infected with culture

Received 1/26/99; accepted 4/16/99.

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[1] This work was supported by Grants R01 CA56041 and P01 CA55261 from the NIH, Grant RPG-96-036-04-CDD from the American Cancer Society, the State of Texas Advanced Technology Program (97-110), and grants from the Gilland Foundation and CaP CURE.

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[3] The abbreviations used are: PI3′K, phosphatidylinositol 3′-kinase; MAPK, mitogen-activated protein kinase; MOI, multiplicity of infection; EGF epidermal growth factor; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; ZVAD, Z-val-ala-Asp(ome)-CH_2F; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling.

media alone. Twenty-four h after infection, cells were changed to serum-free medium. Twenty-four h later, cells were harvested, either with or without stimulation with EGF (50 ng/ml), FCS (10%), or insulin-like growth factor 1 (10 ng/ml) for 10 min. Cells were harvested in lysis solution containing 50 mM HEPES (pH 7.0), 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 100 mM NaF, 10 mM sodium PP_i, 10% glycerol, 1% Triton X-100, 1 mM Na_3VO_4, 10 μM pepstatin, 10 μg/ml aprotinin, 5 mM iodoacetic acid, and 2 μg/ml leupeptin. Western analysis was performed as described previously (14). Immunoblotting was done using antibodies against total and phospho-specific Akt and MAPK (New England Biolabs, Boston, MA), Bcl-2 (Santa Cruz Biotechnology, Santa Cruz, CA), and MMAC1/PTEN (Santa Cruz Biotechnology), followed by horseradish peroxidase-conjugated secondary antibody and detection by chemiluminescence (Amersham, Arlington Heights, IL).

Cell Proliferation Assays. For all assays, cells were grown in media supplemented with 10% FCS. To assay growth, cells (1×10^3) were plated on 96-well plates and infected 18 h later. Cells were then processed using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation assay (Promega Corp., Madison, WI) at the indicated time points thereafter. Statistical comparison of samples was by unpaired Student's *t* test. For cell count analysis, cells (1×10^4) were plated on six-well dishes and infected 18 h later, and viable cell numbers were determined at indicated times thereafter.

Apoptosis Analysis. Cells (1×10^6) were seeded on a 10-cm tissue culture dish and incubated overnight, followed by infection with the indicated viruses or mock treated. Cells were maintained in media supplemented with 10% FCS. Cells were harvested at various times thereafter. For protein analysis, detached and attached cells were collected and lysed in lysis buffer as above. Immunoblotting was done using antibodies against caspase-3 (Transduction Laboratories, Lexington, KY) and caspase-8 (PharMingen, San Diego, CA). Cells for DNA analysis were fixed in 1% paraformaldehyde, stored in 70% ethanol, and analyzed for apoptosis by flow cytometry using the Apo-BrdU kit (Phoenix Flow Systems, San Diego, CA) according to the manufacturer's instructions. For caspase inhibitor experiments, cells were treated with the general caspase inhibitor ZVAD (20 μM; Enzyme Systems Products, Livermore, CA) at 24 and 72 h after infection, followed by harvesting at 96 h after infection. Cells were washed and resuspended in a solution of propidium iodide (50 μg/ml) containing 0.1% sodium citrate and 0.1% Triton X-100 and then analyzed by flow cytometry.

Results

Akt/PKB Regulation. To assess the possible involvement of *MMAC/PTEN* in PI3′K-mediated signal transduction in LNCaP cells, the phosphorylation status of Akt/PKB was determined after infection with adenovirus expressing *MMAC/PTEN*. Forty-eight h after infection with Ad-MMAC, levels of phosphorylation at both activating residues of Akt/PKB, Thr-308, and Ser-473 were markedly decreased in both LNCaP and LNCaP-Bcl-2 cells as compared with cells treated with media alone (mock) or with Ad-DE1, a control adenovirus (Fig. 1*a*). Treatment of the cells with 5 MOI of Ad-MMAC resulted in a decrease of phosphorylation of both activating residues that was >65%, whereas a 25 MOI infection resulted in a decrease of phosphorylation by >95%. The inhibition of Akt/PKB phosphorylation was not due to a decrease in the expression of Akt/PKB because Ad-MMAC-infected cells had similar levels of the protein when compared with control-treated cells (Fig. 1*a*). Furthermore, infection of the cells with control adenovirus (Ad-DE1) or adenovirus expressing p53 (Ad-p53) revealed a significant stimulation of Akt/PKB phosphorylation, further illustrating the ability of *MMAC/PTEN* to down-regulate the phosphorylation of Akt/PKB and its specific biochemical effects. The ability of Ad-MMAC to inhibit the phosphorylation of Akt/PKB was observed in LNCaP and LNCaP-Bcl-2 cells maintained in media supplemented with 10% FCS, as well as in cells acutely stimulated with EGF, insulin-like growth factor 1, or 10% FCS after a period of serum deprivation. MMAC/PTEN expression was as effective as a 30-min pretreatment with wortmannin, a PI3′K inhibitor, at blocking EGF-induced Akt/PKB phosphorylation in LNCaP cells (Fig. 1*a*). The specificity of substrates for MMAC/PTEN was illustrated by its inability to inhibit growth factor-mediated stimulation of phosphorylation of MAPK (ERK1/2). Furthermore, the *in vitro* Akt/PKB kinase activity was significantly decreased by MMAC/PTEN expression and, similar to that observed with glioma cells (14), MMAC/PTEN was more effective than wortmannin in inhibiting the Akt/PKB kinase activity.

The overexpression of Bcl-2 in LNCaP cells did not alter the ability of *MMAC/PTEN* to decrease of the phosphorylation of Akt/PKB. However, a reproducible but higher basal level of Akt/PKB phosphorylation was consistently noted, and a slight increase in the quantity (MOI) of Ad-MMAC was required to produce a similar level of inhibition of Akt/PKB phosphorylation or kinase activity, as compared with the parental LNCaP cells. In addition, the expression of

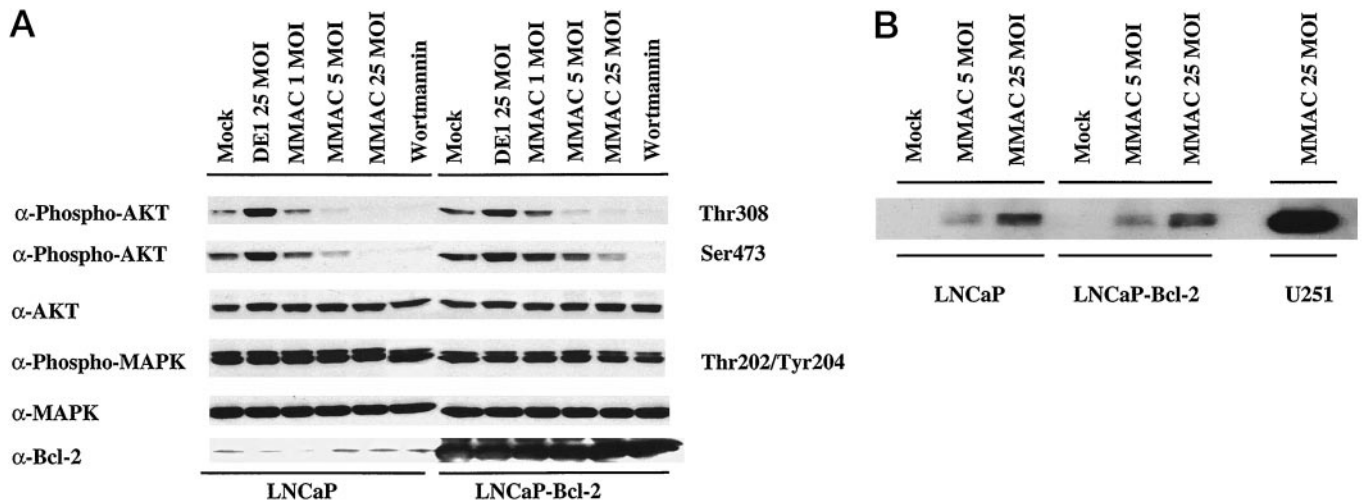


Fig. 1. Effect of MMAC/PTEN expression on signal transduction pathways. *A*, Western blot analysis of EGF-stimulated LNCaP and LNCaP-Bcl-2 cells infected with Ad-MMAC or control adenovirus (Ad-DE1). Forty-eight h after infection, cells were stimulated with EGF (50 ng/ml) for 10 min before harvest. Wortmannin treatment (1 μM) was for 30 min before stimulation. MMAC/PTEN expression inhibits phosphorylation of Akt at both activating residues (Thr-308 and Ser-473) without modifying levels of total Akt protein. MMAC expression did not affect phospho- or total MAPK, or Bcl-2, protein expression. Overexpression of Bcl-2 did not block the inhibition by MMAC/PTEN of Akt phosphorylation. *B*, Western blot analysis of MMAC/PTEN expression in LNCaP and LNCaP-Bcl-2 cells 48 h after infection with media alone (*Mock*) or increasing amounts (MOI) of Ad-MMAC. *Far right lane*, expression of MMAC/PTEN in U251 human glioblastoma cells harvested 48 h after infection with 25 MOI of Ad-MMAC. Gel was loaded with 50 μg of protein per lane. At 48 h, MMAC/PTEN is detectable in LNCaP cells infected with Ad-MMAC, but the amount of MMAC/PTEN protein is significantly less than that seen with identical infection of U251 glioma cells. Comparable levels of expression were seen at later time points. Equal loading was confirmed by immunoblotting with anti-actin antibody (not shown).

MMAC/PTEN did not alter the expression of Bcl-2 protein in either cell type, as assessed from cells using either the endogenous or an exogenous promoter (Fig. 1*a*). At 24 and 48 h after infection, the expression of MMAC/PTEN via the adenoviral construct was significantly reduced in both LNCaP and LNCaP-Bcl-2 cells compared with the levels we observed in U251 glioma cells (Fig. 1*b*). However, higher levels of expression were observed at later time points. A similar increase in expression of p53 via Ad-p53 over the same time course suggests that this difference reflects a difference in the kinetics of expression for LNCaP cells *versus* U251.

Growth Inhibition and Apoptosis. The ability of MMAC/PTEN expression to mediate proliferation and apoptosis of LNCaP cells was assessed by several independent methods. Six days after infection, Ad-MMAC-infected cells were profoundly growth inhibited *versus* mock-infected cells, as determined by a MTS assay that assesses metabolic activity (Fig. 2*a*). LNCaP cells infected with 5 MOI exhibited a 75% decrease in growth, whereas cells infected with 25 MOI showed a 98% inhibition of growth compared with cells mock infected. Ad-MMAC infection resulted in a significantly greater inhibition of growth than that observed with Ad-p53 under the control of the same promoter. Cells infected with Ad-p53 at 5 MOI under the same conditions exhibited a 44% inhibition of growth, whereas 25 MOI produced 78% of the growth of control cells (Fig. 2*a*). Both of these values for Ad-p53 treatment were statistically less effective than treatment with Ad-MMAC at the same MOI (5 MOI, $P < 0.01$; 25 MOI, $P < 0.0001$). Control adenovirus infection exerted a notable toxic effect that resulted in some growth inhibition, but it was significantly less than that observed for either Ad-MMAC or Ad-p53. Similar results were observed when proliferation was determined by counting of viable cells in parallel experiments (Fig. 2*b*).

Comparable studies were performed in parallel on LNCaP-Bcl-2 cells (Fig. 2*c*). At day 6, cells infected with Ad-MMAC at 5 and 25 MOI revealed a 67 and 92% growth inhibition, respectively, compared with mock-infected cells. The growth inhibition produced by Ad-MMAC was again significantly greater than that produced by Ad-p53 at both MOIs, and it appeared that Bcl-2 overexpression slightly decreased the growth-inhibitory effect of p53. As with the LNCaP cells, analysis of growth by cell counts showed a similar differential between the two viruses (Fig. 2*d*).

Because *MMAC/PTEN* and *p53* have both been shown to mediate apoptosis, and because the cell number was decreased below the initial seeding number after infection with 25 MOI of either virus in the proliferation analysis, the ability of the different adenovirus constructs to induce apoptosis in LNCaP and LNCaP-Bcl-2 cells was assessed. TUNEL and propidium iodide staining was performed on cells 4 days after infection (Fig. 3*a*). LNCaP cells infected with 5 MOI of Ad-MMAC showed ~5% of the cells undergoing apoptosis, and about 20% of cells were apoptotic after 25 MOI treatment (Fig. 3*b*). In contrast, Ad-p53 infection resulted in ~5% and 50% of the cells displaying evidence of apoptosis at 5 and 25 MOI, respectively. Ad-p53 induced a greater amount of apoptosis than Ad-MMAC at other time points after infection as well, implying that this discrepancy did not reflect simply a delay in timing of apoptosis induction. Infection of LNCaP cells with control adenovirus at 25 MOI induced a small apoptotic response, but again this was much less than was observed with either Ad-MMAC or Ad-p53. Bcl-2 overexpression largely abrogated the apoptotic effect of MMAC/PTEN or p53 expression in LNCaP cells (Fig. 3, *a* and *c*). However, the cells were nearly as sensitive to the growth-suppressive effects of MMAC/PTEN as parental LNCaP cells. Both LNCaP and LNCaP-Bcl-2 cells infected with Ad-MMAC demonstrated an accumulation of cells in G_1 phase of the cell cycle when compared with mock-infected cells. However, cells infected with control adenovirus also exhibited some G_1 accumulation, thus making it difficult to distinguish whether this arrest was due to MMAC/PTEN expression, adenovirus toxicity, or both.

To further examine the induced apoptosis, the status of different caspases in infected LNCaP and LNCaP-Bcl-2 cells was examined. Caspases are expressed as inactive (proenzyme) precursors in resting cells, which are activated by cleavage at specific internal aspartate residues after apoptosis induction (20). Immunoblots of LNCaP cells infected with 25 MOI of Ad-MMAC or Ad-p53 demonstrated a significant decrease in the quantity of caspase-3 and caspase-8 precursor proteins, indicating that they were both being activated in these cells (Fig. 3*d*). Five MOI of Ad-MMAC failed to induce significant cleavage of either caspase. LNCaP-Bcl-2 cells did not exhibit evidence of caspase processing, consistent with the blockade of apoptosis seen by flow cytometric analysis (Fig. 3, *a* and *c*). To further examine the significance of caspase cleavage, the LNCaP and LNCaP-Bcl-2 cells infected with 25 MOI of Ad-MMAC or Ad-p53 were examined for cell death using propidium iodide staining at various times after treatment with or without ZVAD, a general caspase inhibitor. LNCaP cells infected with Ad-MMAC or Ad-p53 and treated with ZVAD revealed a decrease in the percentage of cells undergoing apoptosis. ZVAD had little effect on the LNCap-Bcl-2 cells, although a significant growth inhibition was still noted in the MMAC/PTEN-expressing cells. These observations imply that the induced MMAC/PTEN growth suppression was largely independent of its apoptosis-inducing ability, whereas for p53, a closer relationship was noted between the two processes.

Discussion

The involvement of *MMAC/PTEN* in prostate carcinoma was initially suggested due to the identification of mutations in the gene in a number of prostate cancer cell lines (8, 9). Additional studies have suggested that similar to gliomas and several other cancers, chromosomal alterations to 10q and mutations affecting *MMAC/PTEN* are predominantly detected in the more advanced grades of prostate carcinomas. Cytogenetic and molecular studies have observed a relatively low rate of loss of heterozygosity in lower grade prostate tumors, although a significant rate of loss of heterozygosity and mutations were found in higher grade and metastatic prostate carcinomas (4, 5, 21–24). Furthermore, a study of human prostate cancer cells xenografts in nude mice observed that although mutations were rare, the majority of xenografts showed reduced or absent expression of *MMAC/PTEN* mRNA and protein product (22). Thus, the loss of function of *MMAC/PTEN*, potentially by several different mechanisms, appears to play a role a prostate cancer tumorigenesis, particularly in cancer progression.

The present study was designed to assess the biological consequences of expression of MMAC/PTEN in prostate cells that are devoid of a functional *MMAC/PTEN* gene product. Acute expression of MMAC/PTEN in LNCaP cells causes inhibition of activation of Akt/PKB, similar to that shown in other cell types and recently in LNCaP cells (13, 14, 25). Furthermore, MMAC/PTEN expression by an adenoviral construct inhibited growth and induced apoptosis in LNCaP cells. However, apoptosis does not appear to account for the majority of the observed growth inhibition and, therefore, suggests that additional growth-regulatory mechanisms are being modulated. This is supported by several observations: (*a*) growth inhibition by 5 MOI of Ad-MMAC is significant (~60–70% of control), whereas minimal apoptosis is observed at this MOI; (*b*) also, LNCaP cells treated with 25 MOI of Ad-MMAC exhibited a decrease in cell growth that could not be adequately accounted for by the apoptotic population, as assessed by TUNEL. *MMAC/PTEN* may also induce

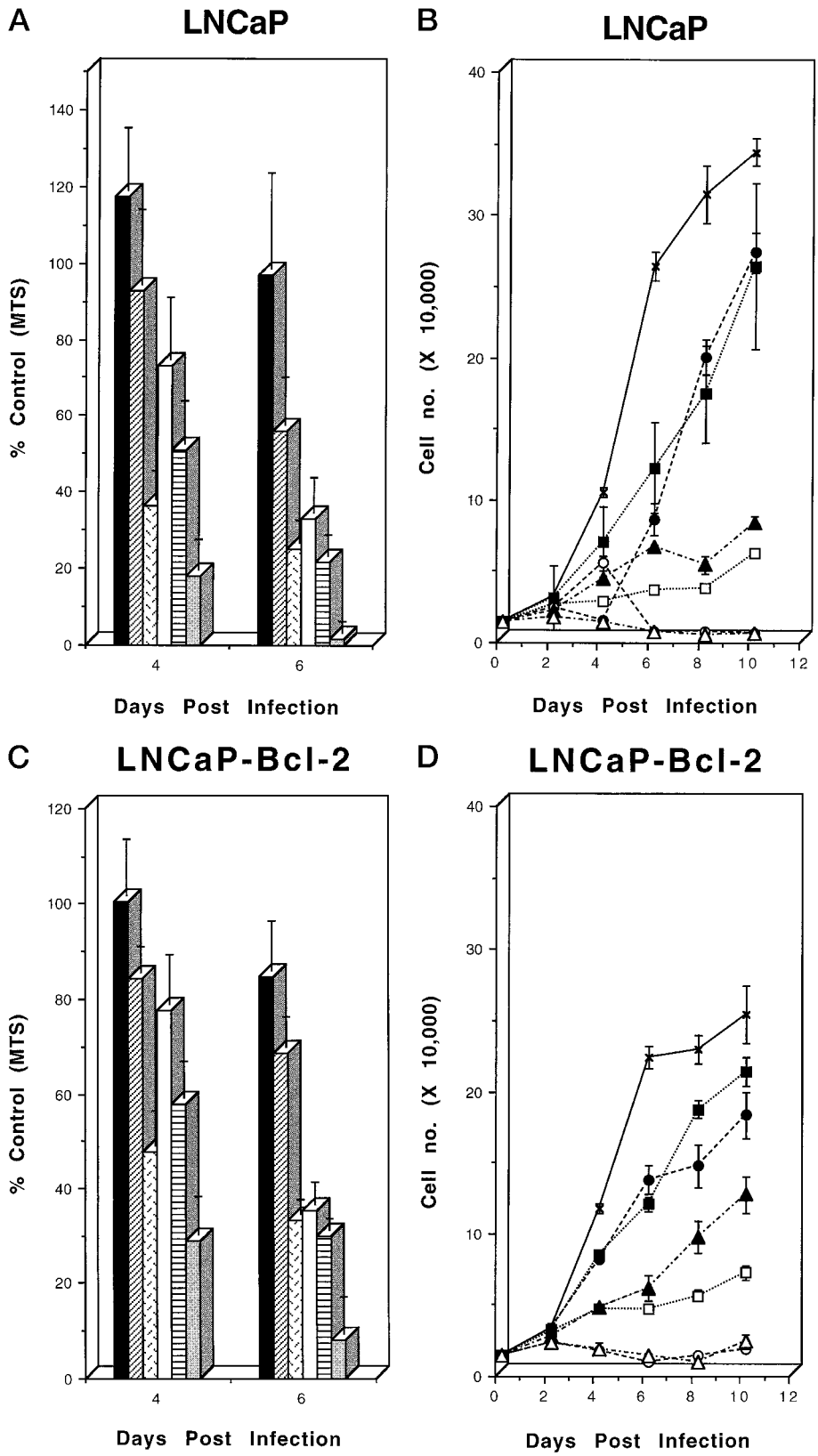
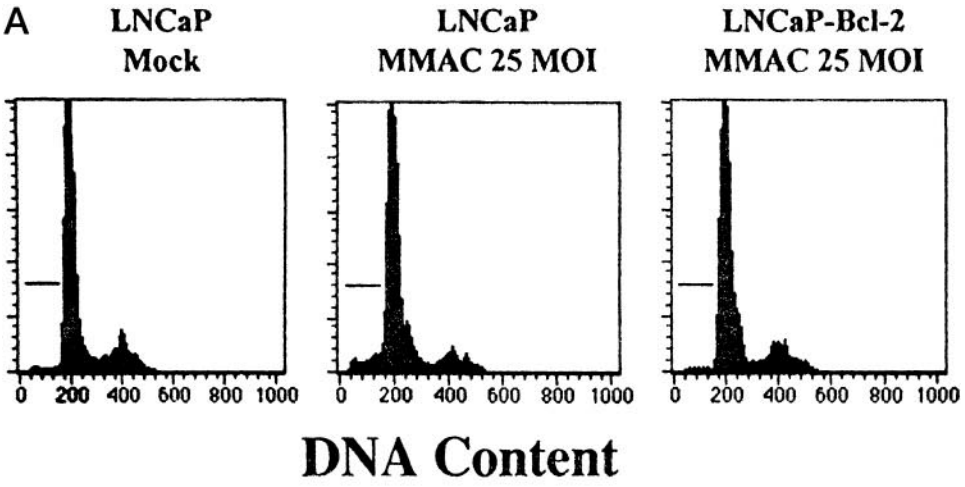
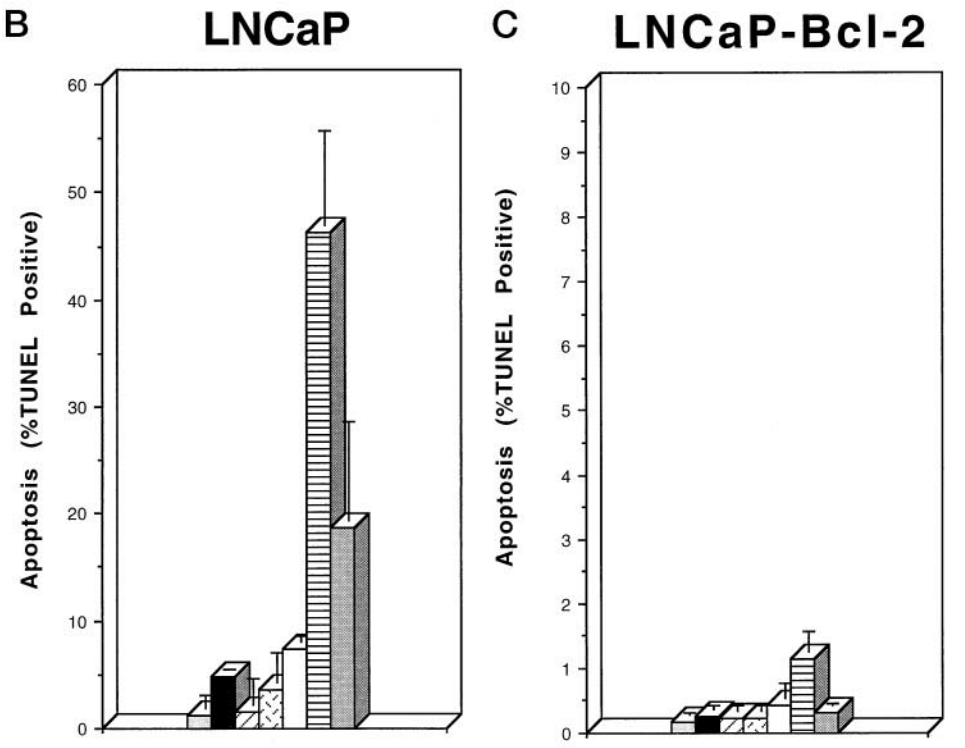


Fig. 2. Effect of MMAC/PTEN and p53 on cell growth. *A*, MTS cell proliferation assay of LNCaP cells 4 days and 6 days after infection with control adenovirus Ad-DE1 (■, 5 MOI; □, 25 MOI), Ad-p53 (▨, 5 MOI; ▤, 25 MOI), or Ad-MMAC (▩, 5 MOI; ▨, 25 MOI). Data are presented as a percentage of MTS value of mock-infected LNCaP cells. *Columns*, means of five replicate samples; *bars*, SD. *B*, cell number of LNCaP cells mock infected (line with Xs) or infected with Ad-DE1 (■, 5 MOI; □, 25 MOI), Ad-p53 (●, 5 MOI; ○,25 MOI), or Ad-MMAC (▲, 5 MOI; △, 25 MOI). *C*, MTS assay of LNCaP-Bcl-2 cells 4 days and 6 days after infection; labels same as in *A*. *D*, cell number of LNCaP-Bcl-2 cells; labels same as in *B*.

nonapoptotic cell death at high expression levels, although viral toxicity in LNCaP cells may also play a significant role; (*c*) additionally, stable overexpression of Bcl-2 in LNCaP cells dramatically decreased *MMAC/PTEN*-induced apoptosis, but it did not substantially diminish the growth-inhibitory effects of *MMAC/PTEN*; and (*d*) LNCaP cells treated with Ad-p53 showed much greater apoptosis of the cellular population (~50–60%) at day 4 but significantly less growth inhibition compared with cells treated with Ad-MMAC. Thus, the expression of MMAC/PTEN in LNCaP prostate cancer cells is capable of producing a marked growth inhibition that appears to be





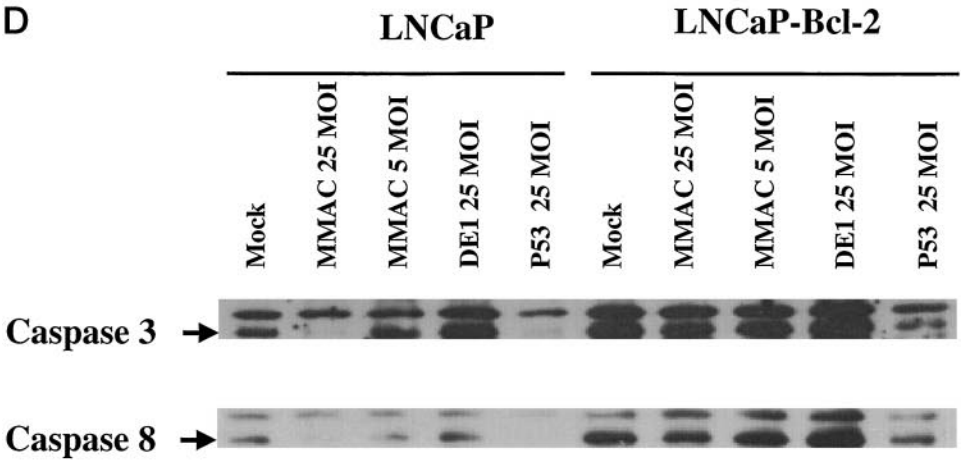


Fig. 3. Assessment of apoptosis induced by MMAC/PTEN in LNCaP cells. *A*, flow cytometric analysis of cells stained for DNA content. Events in area under the *horizontal line* are the sub-G_0, or apoptotic, population. Cells were harvested 4 days after infection. LNCaP cells infected with 25 MOI of Ad-MMAC demonstrated a significant sub-G_0 population, but LNCaP-Bcl-2 cells did not. *B*, quantitation of apoptosis in LNCaP cells 4 days after infection, as assessed by TUNEL, followed by flow cytometric analysis. Cells were mock infected (*speckled column*), or infected with control adenovirus Ad-DE1 (■, 5 MOI; □, 25 MOI), Ad-p53 (▨, 5 MOI; ▤, 25 MOI), or Ad-MMAC (*dashed lines*, 5 MOI; ▧, 25 MOI). In LNCaP cells, 25 MOI of Ad-MMAC induces apoptosis, although not as much as 25 MOI of Ad-p53. Five MOI of neither Ad-MMAC nor Ad-p53 induced significant apoptosis. *Columns*, mean; *bars*, SD. *C*, quantitation of apoptosis in LNCaP-Bcl-2 cells 4 days after infection, as assessed by TUNEL, followed by flow cytometric analysis. Same labels as in *B*; but note that the scale of the *y-axis* is different. Overexpression of Bcl-2 in LNCaP cells blocked apoptosis induction by Ad-MMAC or Ad-p53. *D*, immunoblotting for caspase-3 (*upper*) and caspase-8 (*lower*) in LNCaP and LNCaP-Bcl-2 cells 72 h after infection with indicated adenoviruses. *Arrows*, proenzyme forms of the caspases. LNCaP cells infected with 25 MOI of Ad-MMAC or Ad-p53, but not Ad-DE1, exhibit a marked decrease in the proenzyme form of caspase-3 and caspase-8, consistent with caspase activation and apoptosis. LNCaP-Bcl-2 cells do not show evidence of significant caspase cleavage.

only partially due to its ability to induce apoptosis, although the constitutive expression of MMAC/PTEN appears to be incompatible with the viability and proliferation of LNCaP cells. This conclusion is supported by transfection studies where various forms of *MMAC/PTEN* were inserted into LNCaP cells. Colonies were observed only in cells transfected with mutant forms *of MMAC/PTEN* that affected the phosphatase domain, whereas no colonies were found in cells transfected with a wild-type construct in several independent experiments.

The growth inhibition demonstrated here is intriguing and is under further investigation. There are several possible alternative mechanisms that may be operational. MMAC/PTEN has been shown to modulate the activity of PI3′K-mediated signaling pathways (13–14, 25, 26); therefore, regulation of nonapoptosis-related substrates such as GSK, p70 S6 kinase, or 4E-BP1 may account for the growth inhibition (15). In support of this possibility, the observed inhibition of cell growth as assessed by the MTS assay was greater than that observed when cell number was determined, although both were significant. Interestingly, the expression of MMAC/PTEN from the adenoviral construct at early time points was significantly less in LNCaP cells than that we have observed in U251 glioma cells, although the cell lines demonstrate similar uptake when infected with reporter adenoviruses. This may simply represent a difference in the kinetics of protein metabolism between the two cell lines. However, Wu *et al.* (25) also observed a decrease in expression of several exogenous transient expression constructs in LNCaP cells expressing MMAC/PTEN. We have not observed any decrease in expression of endogenous or stably transfected gene products in the cells (Bcl-2, Akt/PKB, and MAPK). Furthermore, they observed that this effect of expression is also linked to the phosphatidylinositol phosphatase activity of MMAC/PTEN, because activated Akt/PKB appears to rescue cells from repression of gene expression. Therefore, although the mechanism(s) of down-regulation of transient gene expression constructs in LNCaP cells with a functional *MMAC/PTEN* is unknown, these observations suggest that the presence of *MMAC/PTEN* may influence the metabolic and/or transcriptional activities of the cells.

Previously, we have shown that adenoviral transfection of *MMAC/PTEN* into U251 human glioblastoma cells results in a similar inhibition of Akt/PKB activation. However, we did not observe apoptosis in U251 cells unless the cells received an additional apoptosis-inducing stimuli (14). Ad-MMAC also produced a notable, but less pronounced, growth inhibition in U251 cells, which was dramatically less that that observed with adenoviral infection with p53 (19). Growth inhibition without inducing apoptosis was also shown for U87 cells (12, 26). These observations are similar to those shown for fibroblasts that were generated from *MMAC/PTEN*-deficient mice (13). However, breast cancer cells devoid of a functional *MMAC/PTEN* have been shown recently to respond to the expression of functional MMAC/PTEN by undergoing apoptosis (27). The biological differences between the responses of these different cancer cell types does not appear to be due to differential expression of MMAC/PTEN because a number of different expression systems and doses were used. Furthermore, for the breast carcinoma cells, a number of different cell lines exhibited similar biological effects. Thus, *MMAC/PTEN* appears to have disparate effects on different types of cells, although the responses to the expression of MMAC/PTEN in cells devoid of a functional gene product within a particular type of cancer appears to be relatively similar. As such, it will be worthwhile to examine the effect of MMAC/PTEN expression in a variety of cell types to explore its different biological functions, as well as to evaluate its range of therapeutic uses.

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Cancer Res 1999;59:2551-2556.