

# Inhibition of Mycobacterial Infection by the Tumor Suppressor PTEN\*

Received for publication, February 9, 2012, and in revised form, May 21, 2012. Published, JBC Papers in Press, May 21, 2012, DOI 10.1074/jbc.M112.351940

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**Background:** PTEN tumor suppressor is a master cellular regulator but was not previously implicated in pathogenic infection.

**Results:** PTEN expression or pharmacological inhibition of PI3K-Akt-mTOR pathway decreases mycobacterial infection.

**Conclusion:** The lipid phosphatase activity of PTEN suppresses mycobacterial infection by inhibiting PI3K signaling.

**Significance:** Understanding how the genetic context of cells influences their susceptibility to pathogens is important for cancer patient care and for treating pathogenic infection.

The tumor suppressor PTEN is a lipid phosphatase that is frequently mutated in various human cancers. PTEN suppresses tumor cell proliferation, survival, and growth mainly by inhibiting the PI3K-Akt signaling pathway through dephosphorylation of phosphatidylinositol 3,4,5-triphosphate. In addition to its role in tumor suppression, the PTEN-PI3K pathway controls many cellular functions, some of which may be important for cellular resistance to infection. Currently, the intersection between tumorigenic signaling pathways and cellular susceptibility to infection is not well defined. In this study we report that PTEN signaling regulates infection of both noncancerous and cancerous cells by multiple intracellular mycobacterial pathogens and that pharmacological modulation of PTEN signaling can affect mycobacterial infection. We found that PTEN deficiency renders multiple types of cells hyper-susceptible to infection by *Mycoplasma* and *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG). The lipid phosphatase activity of PTEN is required for attenuating infection. Furthermore, we found mycobacterial infection activates host cell Akt phosphorylation, and pharmacological inhibition of Akt or PI3K activity reduced levels of intracellular infection. Intriguingly, inhibition of mTOR, one of the downstream components of the Akt signaling and a promising cancer therapeutic target, also lowered intracellular Bacillus Calmette-Guérin levels in mammary epithelial cancer MCF-7 cells. These findings demonstrate a critical role of PTEN-regulated pathways in pathogen infection. The relationship of PTEN-PI3K-Akt mTOR status and susceptibility to mycobacterial infection suggests that the interaction of mycobacterial pathogens with cancer cells may be influenced by genetic alterations in the tumor cells.

The tumor suppressor PTEN plays critical roles in cell growth, migration, and death (1–5). PTEN is frequently

mutated or deleted in various human cancers and is an important determinant of tumorigenesis (5–7). The PTEN protein is a phosphatase for the second messenger phosphatidylinositol 3,4,5-triphosphate, which is required for activation of the Akt/PKB kinase in the PI3K-Akt pathway (1, 5, 8). The PI3K-Akt pathway promotes cell metabolism, survival, and proliferation (9). One of the downstream effectors of the pathway, the mTOR-Raptor kinase complex, is the master regulator of cellular metabolism (10). As such, genetic mutation of PTEN and other components of this pathway have significant impact on pathogenesis of cancer and other diseases.

The PI3K-Akt pathway has also been implicated in infectious diseases and innate immunity. Mounting evidence supports the involvement of Akt in phagocytosis, intracellular bacterial infections, LPS tolerance, production of inflammatory cytokines and mediators, and migration during macrophage-mediated innate immunity, thus establishing a pivotal role for this enzyme in the functional activation of macrophages (11). Furthermore, it was found that intracellular survival and growth of certain phagocytosed bacteria in macrophages are controlled by a kinase network, of which Akt acts as a master regulator by controlling multiple host pathways (12).

Because the Akt pathway plays a role in both cancer development and interactions of pathogens with host cells, it is possible that these two events influence each other, especially during attempts to treat cancer with infectious biotherapies. One successful use of a bacterial pathogen to treat cancer is the use of an attenuated strain of *Mycobacterium bovis*, BCG,<sup>2</sup> to treat superficial bladder cancer. Despite more than 30 years of clinical experience with intravesical BCG for bladder cancer, no markers exist to predict which patients will respond to therapy. This is due in part to the fact that its mechanism of action is not well understood. Direct and indirect immune mechanisms have been hypothesized to play a role (13, 14) as have direct cytotoxic effects of BCG (15). Whatever the mechanism of action, it does seem clear that BCG interaction with tumor cells, leading to internalization and processing of the mycobacterium, plays a

\* This work was supported, in whole or in part, by National Institutes of Health Grant R01CA113890 (to X. J.). This work was also supported by the Geoffrey Beene Cancer Research fund (to M. S. G. and X. J.).

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<sup>2</sup> The abbreviations used are: BCG, Bacillus Calmette-Guérin; MEF, mouse embryonic fibroblasts.

crucial role in activation of BCG-mediated anti-tumor activity (16–19).

In this study we demonstrate that the activity of the PI3K-PTEN-Akt pathway determines the susceptibility of cells to infection by mycobacteria, including BCG, suggesting that tumor cell genetic characteristics may be important determinants of the BCG-tumor cell interaction.

## EXPERIMENTAL PROCEDURES

**Antibodies**—Antibodies against S6 (5G10, #2217), S6K (49D7, #2708), p-S6K (Thr389) (108D2, #9234), Akt (#9272), p-Akt (Ser473) (#9271), and p-Akt (Thr308) (#2965) were from Cell Signaling Technology. PTEN antibody (clone 6H2.1) was from Cascade BioScience. Antibodies against PCAF (E-8), actin (C-2), and GFP (B-2) were from Santa Cruz Biotechnology, Inc. Tubulin antibody (DM1A) was from Calbiochem.

**Detection of Mycoplasmas with DAPI**—Cells were fixed in 3.7% formaldehyde for 10 min, permeabilized with 0.5% Triton X-100 for 5 min, and stained with DAPI at 1  $\mu\text{g}/\text{ml}$ . On adding DAPI to tissue culture cells, it is rapidly taken up into cellular DNA yielding highly fluorescent nuclei and no detectable cytoplasmic fluorescence. If the cells are contaminated with *Mycoplasma*, characteristic discrete fluorescent foci are readily detected over the cytoplasm and sometimes in intercellular spaces. Infection by mycoplasmas was further confirmed by detecting the presence of conserved mycoplasmal enzymes with a MycoAlert™ kit (Lonza Bioscience).

A detailed analysis from the institutional facility indicates that the source of *Mycoplasma* that used in our experiments is a mixture mainly containing the following two species, *Mycoplasma orale* and *Mycoplasma fermentans*. *M. orale* was detected by PCR with primers of 5'-ACACCATGGGAGCTGTGTAAT-3' and 5'-CTTCTTCGACTTTCAGACCCAAGGCAT-3', which gives a product of 423 bp in size. *GAPDH* was used as internal control with primers of 5'-ATTTGGCCGTA-TTGGGCGCCTG-3' and 5'-CCCGGCCTTCTCCATGG-TGG-3' producing a 298-bp band.

**Cell Lines**—PC3, HeLa, MCF-7, UM-UC-3, and MGHU4 were obtained from the American Type Culture Collection (ATCC) and were cultured according to ATCC instructions. All cell lines were tested for their authenticity by cytogenetic analysis. Mouse epithelial fibroblasts (MEFs) of both *pten*<sup>+/+</sup> and *pten*<sup>-/-</sup> were kindly provided by Dr. Pier Paolo Pandolfi and cultured and tested as previously described (2).

**Transfection, PTEN RNAi Constructs, and Establishment of Stable Cell Lines**—Cell culture transfection was performed using Lipofectamine 2000 from Invitrogen. The shRNA constructs against human PTEN generated using pLKO.1 vector were bought from Sigma. The PTEN DNA sequences used in the RNAi construct were 5'-AGGCGCTATGTGTATTAT-TAT-3', 5'-CCACAGCTAGAAGCTTATCAAA-3', 5'-CTAGAACTTATCAAACCTTT-3', 5'-CGTGCAGATAATGACAAGGAA-3', and 5'-CCACAAATGAAGGGATATAAA-3'. Stable cell lines were established by infection of cells with pLKO.1 lentivirus. Puromycin selection at 0.6  $\mu\text{g}/\text{ml}$  was carried out 24 h after removal of virus.

**BCG and Infection**—GFP-expressing BCG was created by transforming *M. bovis* Calmette-Guérin Pasteur strain with

pYUB921 (expresses GFP and kanamycin resistance) (20). BCG-GFP was grown at 37 °C in Middlebrook 7H9 media supplemented with 10% albumin, dextrose, saline, 0.5% glycerol, and 0.05% Tween 80 and in the presence of 20 mg/ml kanamycin. To create a stock for infection, BCG-GFP was grown to mid-log phase ( $A_{600}$  0.4–0.6), washed twice in phosphate-buffered saline (PBS) with 0.05% Tween 80, resuspended in PBS with 25% glycerol, and stored at –80 °C. To measure final bacterial concentration, an aliquot was thawed, and serial dilutions were plated on 7H10 plates in the presence of 20 microgram/ml kanamycin. Cells were plated a day before infection in antibiotic-free media so as to reach 90% confluence on the day of infection. BCG-GFP was thawed and diluted in antibiotic-free media to achieve a multiplicity of infection of 10:1. Plates were incubated at 37 °C for the specified time period and then washed 3 times with PBS and 3 times with antibiotic-containing media (100 units/ml penicillin G and 100  $\mu\text{g}/\text{ml}$  streptomycin). Cells were further incubated for indicated time before being imaged or detached using trypsin and resuspended in PBS for analysis by flow-cytometry.

**Inhibitors**—All inhibitors were used at concentrations reported to inhibit their kinase target. Wortmannin and rapamycin (Sigma) were used at a concentration of 100 nM to inhibit PI3K and mTOR, respectively. Akt was inhibited with Akti-1 (Calbiochem) at 10  $\mu\text{M}$  or Akti-2 (Merck) at 2  $\mu\text{M}$ . Inhibitors were added after infection and removal of extracellular BCG. To test if PI3K-Akt pathway is involved in BCG entry, cells were also pretreated with inhibitors 2 h before infection.

**Immunostaining**—To detect mitochondria, cells grown on glass coverslips in culture medium were first treated with 500 nM of MitoTracker Red CMXRos for 45 min before being fixed, permeabilized, and stained with DAPI. Polyribosome was stained with an antibody against S6 ribosomal protein.

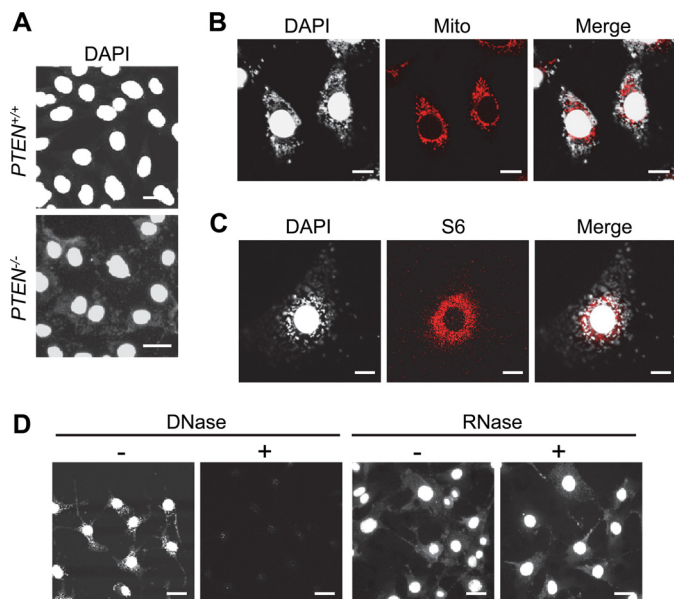
**Flow Cytometry**—Samples were acquired on an LSR II flow cytometric device (BD Biosciences) using the FACS DiVa software (BD Biosciences) according to the manufacturer's instructions. Data analysis was performed with the FlowJo software package (Tree Star).

**Nuclease Digestion**—Cells were fixed in methanol for 2 min at –20 °C, rinsed in PBS and incubated in either DNase I (100  $\mu\text{g}/\text{ml}$  in PBS and 5 mM  $\text{MgCl}_2$ , RNase-free) or RNase A (100  $\mu\text{g}/\text{ml}$  in PBS, DNase-free) for 2 h at 25 °C. After several washes in PBS, cells were stained with DAPI as described above.

## RESULTS

**Absence of PTEN Expression Confers Susceptibility to Mycoplasma Infection**—This work originated from a serendipitous observation in the course of our investigation of the PTEN pathway. With fluorescent microscopy, we observed that MEFs deficient in PTEN had unusually high levels of DAPI-stained structures in the cytoplasm (Fig. 1A). These cytosolic DAPI-positive particles did not co-localize with mitochondrial or ribosomal stains, indicating that they are not clusters of mitochondria or polyribosomes, the two major cytosolic nucleic acid-containing structures (Fig. 1, B and C). The DAPI stained structures are sensitive to DNase but not RNase digestion, indicating that the DAPI signal is from cytosolic DNA (Fig. 1D). We subsequently found that these cytosolic DAPI-positive struc-

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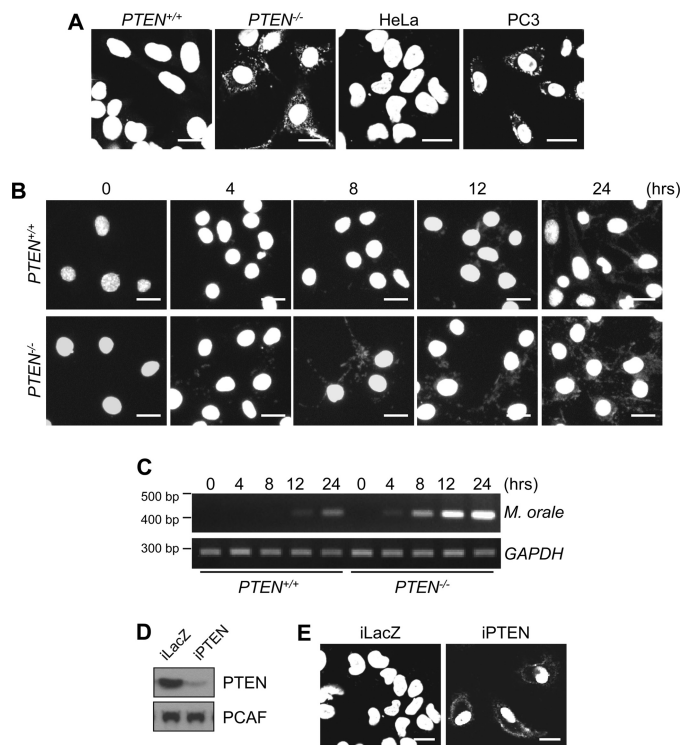


**FIGURE 1. Cytosolic DAPI signal detected in *pten*<sup>-/-</sup> MEFs.** A, DAPI-positive signal was detected in the cytoplasm of *pten*<sup>-/-</sup> MEFs but not wild-type MEFs. Scale bar = 20  $\mu$ m. B, cytosolic DAPI signal was not from mitochondria. Mitochondria were detected by MitoTracker Red (*Mito*) as described under "Experimental Procedures." Scale bar = 10  $\mu$ m. C, the cytosolic DAPI signal was not from polyribosomes. Polyribosomes were stained with a primary antibody against S6 ribosomal protein and then a Cy3 conjugated secondary antibody. Scale bar = 10  $\mu$ m. D, the cytosolic DAPI signal can be removed by DNase but not RNase. Cytosolic DAPI-positive *pten*<sup>-/-</sup> MEFs were treated with either DNase or RNase as described under "Experimental Procedures" and then subjected to DAPI staining. Scale bar = 20  $\mu$ m.

tures were infected *Mycoplasma* (the laboratory had just suffered a *Mycoplasma* infection then).

The tight correlation of the *Mycoplasma* with the PTEN status of cells prompted us to further investigate the potential relationship between PTEN function and *Mycoplasma* infection. First, we infected a battery of cell lines with culture medium containing *Mycoplasma*. These cell lines include wild-type MEFs and HeLa cells (both PTEN-positive) as well as PTEN-null MEFs and human prostate cancer cells PC3 (both PTEN-negative). Indeed, compared with PTEN-positive cells, PTEN-negative cells were hypersusceptible to infection with *Mycoplasma* (Fig. 2A). Second, in a time-course experiment, we subjected both wild-type MEFs and PTEN-null MEFs to *Mycoplasma* infection and monitored intracellular *Mycoplasma* using DAPI-staining at different time points. PTEN-null MEFs showed detectable intracellular *Mycoplasma* after 8 h, whereas wild-type MEFs showed similar levels of infection only after more than 24 h (Fig. 2B). This was confirmed by PCR with primers specific to *M. orale* (Fig. 2C). To further validate that PTEN status influences susceptibility to *Mycoplasma* infection, we knocked down PTEN by shRNA in HeLa cells and measured *Mycoplasma* infection (Fig. 2, D and E). As expected, HeLa cells with PTEN knockdown showed hypersusceptibility to *Mycoplasma* infection compared with cells that had received a LacZ shRNA control. Taken together, these results indicate that defective PTEN function results in higher susceptibility of host cells to *Mycoplasma* infection.

**Suppression of Intracellular Mycoplasmas Depends on Lipid Phosphatase Activity of PTEN**—Next, we tested whether overexpression of PTEN can reduce intracellular *Mycoplasma* lev-



**FIGURE 2. Absence of PTEN expression confers susceptibility of cells to *Mycoplasma* infection.** A, PTEN-null cells are prone to infection by *Mycoplasma*. PTEN-positive cells (wild-type MEFs and HeLa cells) and PTEN-null cells (*pten*<sup>-/-</sup> MEFs and PC3 cells) were infected with culture medium containing *Mycoplasma* and then tested by DAPI staining as described under "Experimental Procedures." B, *pten*<sup>-/-</sup> MEF cells were infected by *Mycoplasma* more quickly than the wild-type counterpart. Both *pten*<sup>-/-</sup> and wild-type MEFs were infected with *Mycoplasma*. Infection was followed by DAPI staining at the indicated time points. C, infection was also detected by PCR using primers specific for *M. orale*. D, Western blot shows the effect of knockdown of PTEN by shRNA (*iPTEN*) in HeLa cells. LacZ shRNA (*iLacZ*) was used as negative control. A PCAF blot serves as the loading control. E, PTEN knockdown but not control knockdown makes HeLa *Mycoplasma* infection-prone. *Mycoplasma* infection was monitored by DAPI staining. Scale bar = 20  $\mu$ m.

els and whether the enzymatic activity of PTEN is required. *Mycoplasma*-infected PTEN-deficient PC3 cells were transfected with either GFP-tagged wild-type PTEN or lipid phosphatase inactive mutants (C124S or G129E mutant). As expected, in the PTEN-negative PC3 cells, Akt is constitutively activated, shown by high levels of phosphorylation at serine 473 (Fig. 3A). Overexpression of wild-type PTEN, but not C124S or G129E PTEN, suppressed Akt activation (Fig. 3A). Importantly, we found that restoration of PTEN expression eliminated intracellular *Mycoplasma* and that this role was dependent on PTEN lipid phosphatase activity, as both the C124S and G129E mutants failed to do so (Fig. 3B). Recent evidence suggests that PTEN can also regulate various cellular processes with its protein phosphatase activity, although it is not clear what its physiological protein substrates are (3, 4, 21–23). Because the G129E mutant is still functional as a protein phosphatase (3), failure of the G129E mutant to suppress intracellular *Mycoplasma* indicates that the protein phosphatase activity of PTEN is not sufficient for this function.

**PTEN Inhibits Intracellular BCG Growth in MCF-7 Cells**—*Mycoplasmas* are cell wall deficient bacteria that can exist either extracellularly or within the cytoplasm of host cells (24). We sought to understand whether PTEN deficiency confers



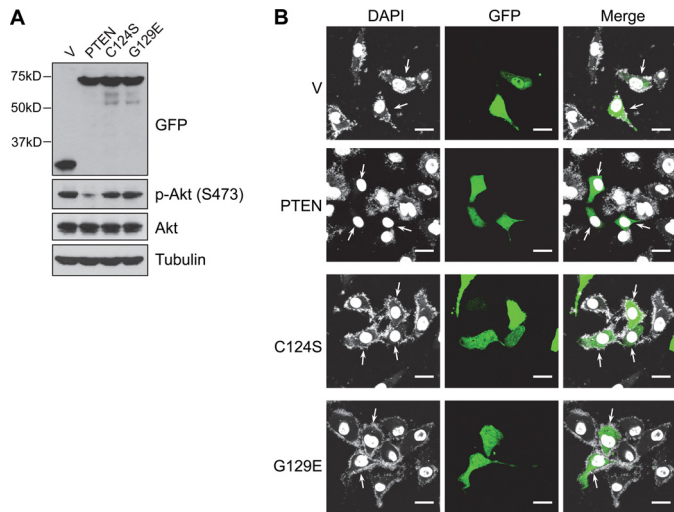
susceptibility to other intracellular bacterial pathogens. For this purpose, we used *M. bovis* BCG, an attenuated strain of *M. bovis* that was derived by prolonged *in vitro* passage. Pathogenic mycobacteria are intracellular pathogens that replicate and survive within host cells, usually professional phagocytes such as macrophages and dendritic cells.

We first infected MCF-7 mammary epithelial cancer cells with BCG carrying an episomal plasmid expressing green fluorescent protein (GFP). This fluorescent marker allowed easy quantitation of intracellular BCG by fluorescent microscopy and flow cytometry. We found that shRNA knockdown of PTEN (Fig. 4A) enhanced BCG infection in MCF-7 cells, as

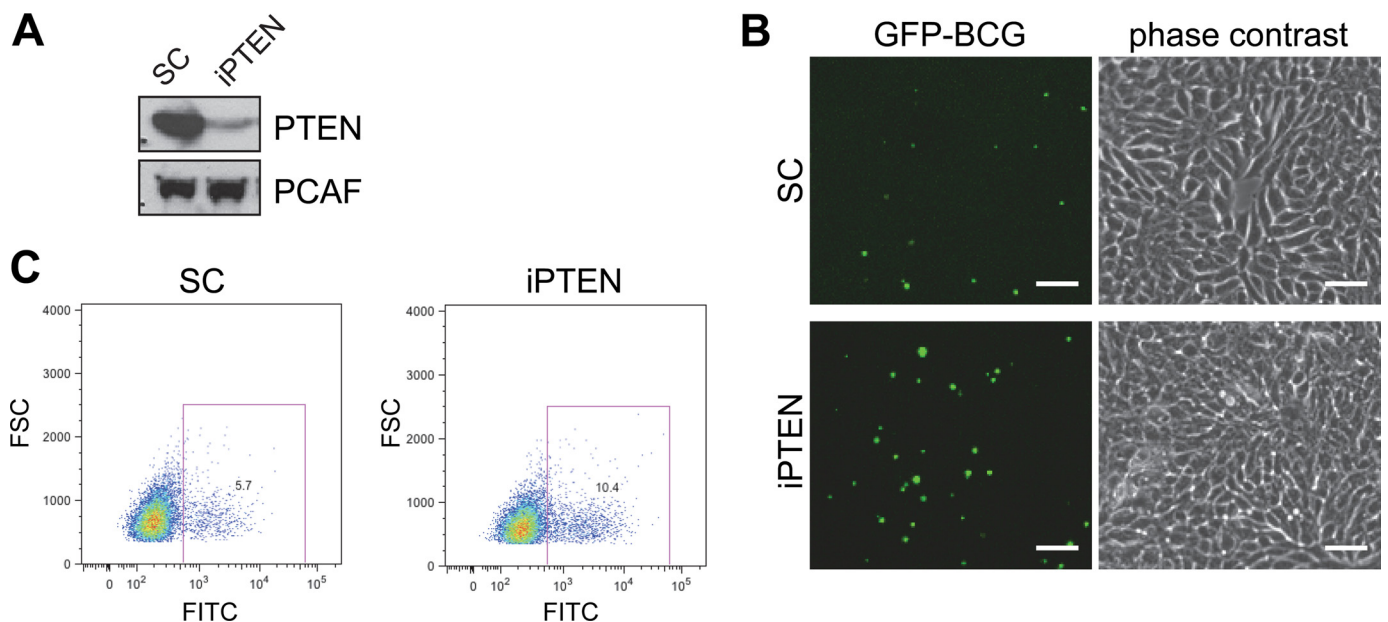
reflected by the number and intensity of GFP foci inside MCF-7 cells determined by fluorescence microscopy and flow cytometry (Fig. 4, B and C).

**PTEN Inhibits Intracellular Pathogen Growth in MCF-7 Cells through Suppressing PI3K-Akt-mTOR Pathway**—PTEN is a predominant negative regulator of the PI3K-Akt pathway. To test whether the effect of PTEN knockdown on intracellular growth is mediated by activation of the PI3K-Akt pathway, we examined the effect of mycobacterial infection on host cell Akt activation in MCF-7 cells. As shown in Fig. 5, both BCG and *Mycoplasma* activated Akt as early as 1 h after infection (Fig. 5A). To test whether Akt activation is required for mycobacterial infection, we treated infected cells with small molecule inhibitors of Akt, PI3K, and mTOR. The efficacy of these inhibitors was verified by measuring their effect on inhibition of Akt and S6K phosphorylation (Fig. 5B). Indeed, inhibition of Akt phosphorylation and PI3K (wortmannin) reduced BCG infection in infected cells, as manifested by reduced number and size of intracellular GFP-BCG foci (Fig. 5, C and D). Surprisingly, we found that inhibition of mTOR (rapamycin), one of the downstream components of the Akt signaling and a promising cancer therapeutic target, also conferred resistance to BCG infection (Fig. 5, C and D). This function of mTOR is apparently distinct from the proposed role of the Akt in pathogenic infection, because 1) the endocytic and phagocytic function of Akt does not require mTOR, and 2) inhibition of mTOR did not ablate Akt activation in MCF-7 cells (Fig. 5B) and in many cell types even leads to a strong feedback activation of Akt, as demonstrated previously (25).

To address whether the PI3K-Akt pathway also promotes BCG entry, we compared the degree of BCG infection by treating MCF-7 cells with the inhibitors either before or after BCG infection. We did not observe any obvious change in percentages of infected cells (Fig. 5D), indicating phosphatidylinositol-

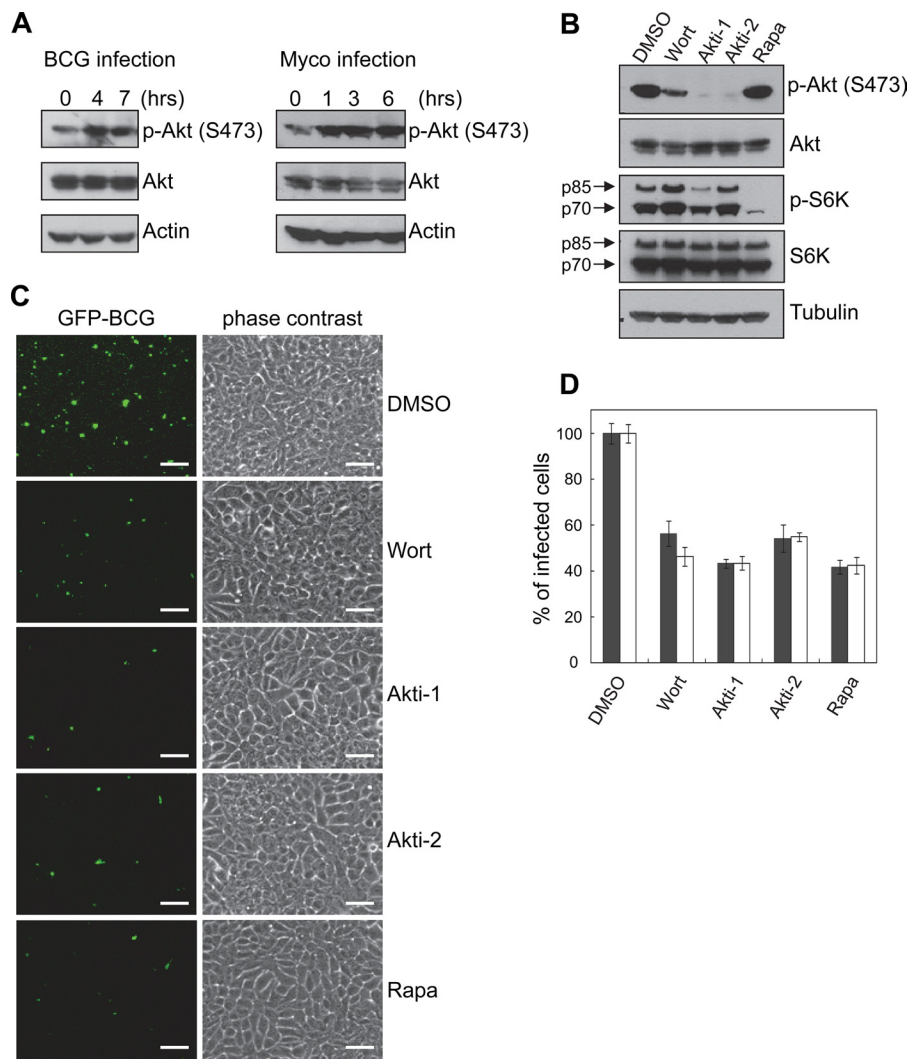


**FIGURE 3. The lipid phosphatase activity of PTEN is required for suppression of intracellular *Mycoplasma*.** PC3 were infected with *Mycoplasma* and transiently transfected with empty GFP vector (V) or GFP tagged PTEN, PTEN C124S, or PTEN G129E. Intracellular mycoplasmas were evaluated 3 days after by DAPI staining. *A*, a Western blot shows equal levels of expression of PTEN and its derivatives and their ability to inhibit Akt phosphorylation. *B*, wild-type PTEN, but not lipid phosphatase-dead mutants, suppressed intracellular *Mycoplasma* (indicated by arrows). Scale bar = 20  $\mu$ m.



**FIGURE 4. PTEN inhibits BCG infection in MCF-7 cells.** MCF-7 cells, harboring either scrambled shRNA knockdown (SC) or PTEN knockdown (iPTEN) (A) were infected with GFP-BCG at multiplicity of infection of 10 for 5 h. Extracellular bacteria were washed away, and cells were incubated for 72 h before intracellular bacterial levels were determined by confocal microscopy (B) or flow cytometry (C). Scale bar = 50  $\mu$ m. FSC, forward scatterer.

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**FIGURE 5. PTEN inhibits pathogen infection in MCF-7 cells through suppressing PI3K-Akt-mTOR pathway.** *A*, infection with both *Mycobacterium* and BCG leads to Akt activation in MCF-7 cells. MCF-7 cells were infected with either BCG or *Mycobacterium* (*Myco*) and harvested at different time points. Level of p-Akt at serine 473 was measured by immunoblot. *B*, Western blotting shows the efficacy of PI3K-Akt-mTOR pathway inhibition by different inhibitors. MCF-7 cells were infected with GFP-BCG. Extracellular bacteria were removed, and cells were further treated with different inhibitors for 72 h before harvest. The efficacy of inhibition was evaluated by suppression of phosphorylation of Akt and S6K. *C* and *D*, inhibition of PI3K-Akt-mTOR pathway blocks survival and/or proliferation but not entry of BCG in MCF-7. Inhibitors were added to cells either after (*black column*) or before (*white column*) infection. Intracellular BCG levels were determined by fluorescent microscopy (*C*) and flow cytometry (*D*). Results are averaged from three independent experiments, and infected cells in DMSO control was considered as 100%. Scale bar = 50  $\mu$ m.

3,4,5-triphosphate signal is not involved in pathogen entry under this condition.

**Effect of PTEN Deficiency on BCG Infection in Bladder Cancer Cells**—To extend our findings to a tumor cell type that may encounter BCG during the course of cancer therapy, we also determined the susceptibility of bladder cancer cells with altered PTEN levels to infection with BCG. We infected bladder cancer cell lines UM-UC-3 and MGHU4 with GFP-expressing BCG and measured the proportion of infected (GFP-positive) cells by flow cytometry. Western blots revealed that UM-UC-3 cells lack PTEN expression, whereas MGHU4 cells possess readily detectable PTEN expression (Fig. 6A). After 22 h of infection there was a dramatic difference between the two cell lines in their susceptibility to BCG infection. MGHU4 was highly resistant to infection, with less than 2% of the population being GFP-positive, whereas UM-UC-3 was susceptible to infection with more than 50% of the cells being GFP-positive

(Fig. 6B). To assess whether loss of PTEN is sufficient to render MGHU4 cells susceptible to BCG infection, we knocked down PTEN in MGHU4 by shRNA (Fig. 6C). PTEN knockdown increased the fraction of infected cells by ~2-fold compared with control shRNA (Fig. 6D). It should be noted that the absolute level of infection in MGHU4 cells upon PTEN knockdown did not approach that observed in UM-UC-3 cells. This might be due to incomplete PTEN knockdown (Fig. 6C), and it is also likely that other factors might contribute to the difference of these two cell lines in susceptibility to BCG infection. Taken together, our results indicate a causal relationship between the PTEN status of epithelial cells and their permissiveness for intracellular pathogens.

## DISCUSSION

**Loss of PTEN Confers Susceptibility to Intracellular Infection Across Pathogen and Cell Types**—This study indicates that a competent PTEN protein is not only critical for tumor suppres-

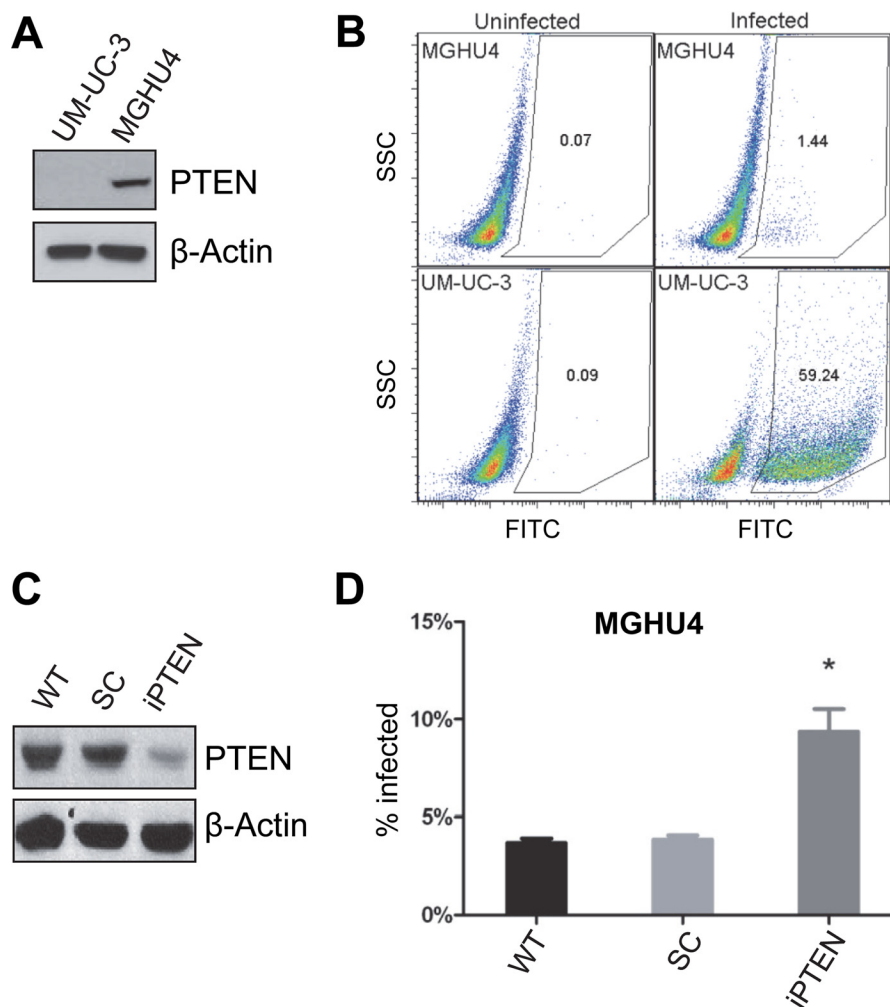


FIGURE 6. **PTEN inhibits BCG infection in bladder cancer cells.** *A*, a Western blot shows the levels of PTEN and actin in UM-UC-3 and MGHU4. *B*, shown are flow cytometry plots of UM-UC-3 (top panels) or MGHU4 cells (bottom panels) either uninfected (left panels) or infected with BCG-GFP (right panels) for 22 h. The x axis plots GFP fluorescence intensity on a log scale and the numbers within each quadrant are the percent of GFP-positive cells within the indicated gate. *C* and *D*, PTEN shRNA increases infection in MGHU4 cells when compared with wild-type or non-targeting shRNA transfected cells. Knockdown of PTEN in MGHU4 was confirmed by Western blot (*C*). *SC*, scramble shRNA; *iPTEN*, PTEN shRNA. \*,  $p < 0.05$ , compared with scramble shRNA. *SSC*, side scatter.

sion but also for resisting infection of mammalian cells by BCG and *Mycoplasma*, two pathogens with distinct pathogenic strategies. Our data also indicate that infection with BCG or *Mycoplasma* activates Akt, indicating that loss of PTEN may promote infection by mimicking the natural pathogenic strategy used by these organisms. The mechanisms that mediate the effect of PTEN loss on bacterial infection remain to be defined and could be the consequence of enhanced bacterial intracellular proliferation, intracellular survival, or a combination.

Previous reports suggested a role of Akt in mediating infection of epithelial cells and macrophages by mycobacteria and *Salmonella* (12, 26, 27). Although the mechanisms by which Akt functions to promote bacterial entry, intracellular proliferation, or survival has not been defined, hypotheses have been raised to explain this phenomenon. By activating p21-activated kinase 4 (PAK4) and downstream small G protein RAC1, Akt modulates actin cytoskeleton and phagocytosis, thus possibly affecting bacterial entry (28, 29); by accelerating cycling of RAB14 small G protein, Akt delays phagosomal-lysosomal fusion, thus likely enhancing intracellular survival of bacteria (30, 31). All these functions of Akt do not involve mTOR, mak-

ing our finding that rapamycin can suppress BCG infection in MCF-7 cells highly intriguing. Inhibition of mTOR by rapamycin can promote autophagy. To test if autophagy contributes to the effect of rapamycin on BCG infection, we knocked down the autophagy-essential gene ATG7 in MCF-7 to abrogate autophagy. However, ATG7 deficiency does not affect BCG infection in MCF-7 (data not shown), suggesting autophagy is not responsible for our observation at least under this context.

**Pathogenic Infection and Cancer**—The fact that PTEN-deficient cells are broadly sensitive to bacterial infection has potential implications for clinical oncology. This study suggests that certain tumor cells, especially those with PTEN mutation or PI3K/Akt activation, might be more susceptible to infection by certain intracellular bacteria. To date, whether cancer cells are in general more vulnerable to pathogenic infection and whether pathogenic infection could affect cancer development are underexplored areas that have potential clinical significance.

The PTEN-PI3K pathway is a commonly mutated pathway in human cancer. Consequently, the kinases in this and related pathways are being aggressively pursued as targets for therapeutic intervention. Our finding that loss of PTEN with conse-



quent activation of the PI3K pathway confers intrinsic susceptibility to infection suggests that these inhibitors may have complex effects on infectious risk in patients. Inhibitors of mTOR such as rapamycin and sirolimus are potent immunosuppressants due to their effects on T cell proliferation. However, our results indicate that small molecule inhibitors of the PI3K pathway may actually promote resistance to infection in epithelial cells and possibly macrophages. Our results also have potential implications for BCG therapy of Carcinoma *in situ* of the bladder. Our results demonstrate that the genetic background of a bladder cancer cell may affect its susceptibility to BCG infection, a variable that could determine response to BCG therapy. Further elucidation of the molecular determinants of bladder cancer cell susceptibility to BCG infection has the potential to reveal predictors of BCG response, none of which are presently available for clinical use.

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