Cardiac hypertrophy is a complex process involving the coordinated actions of many genes. In a high throughput screen designed to identify transcripts that are actively translated during cardiac hypertrophy, we identified a number of genes with established links to hypertrophy, including those coding for Sp3, c-Jun, annexin II, cathepsin B, and HB-EGF, thus showing the general utility of the screen. Focusing on a candidate transcript that has not been previously linked to hypertrophy, we found that protein levels of the tumor suppressor PTEN (phosphatase and tensin homologue on chromosome ten) were increased in the absence of increased messenger RNA levels. Increased PTEN expression by recombinant adenovirus in cultured neonatal rat primary cardiomyocytes caused cardiomyocyte apoptosis as evidenced by increased caspase-3 activity and cleaved poly(ADP-ribose) polymerase. Expression of PTEN was also able to block growth factor signaling through the phosphatidylinositol 3,4,5-triphosphate pathway. Surprisingly, expression of a catalytically inactive PTEN mutant led to cardiomyocyte hypertrophy, with increased protein synthesis, cell surface area, and atrial natriuretic factor expression. This hypertrophy was accompanied by an increase in Akt activity and improved cell viability in culture.

Cardiac hypertrophy is an adaptive response of the heart to both intrinsic and extrinsic stimuli. Causes of hypertrophy include hypertension, myocardial infarction, endocrine disease, and mutations in components of the contractile apparatus that impair pump function (1). Initial hypertrophy may be beneficial, alleviating chamber wall stress and increasing cardiac output, but chronic stimulation can lead to cardiomyopathy, decompensation, and failure. The molecular pathways invoked during this pathological progression are now being defined, but it is already clear that no single effector is responsible for the observed alterations in cardiac gene expression, apoptosis, and fibrosis. In addition to mechanical stresses, many recently identified ligands, including adrenergic signals, peptide growth factors, and cytokines can evoke cardiomyocyte hypertrophy. Under certain conditions, the same factors that induce cardiomyocyte hypertrophy, such as insulin growth factor-1 (IGF-1) and calcineurin (2, 3), can also influence cell survival. Hence, extrinsic stimuli on the cardiomyocyte surface are no longer thought to necessarily evoke a linear signaling cascade to the nucleus or contractile machinery but rather to feed into a “signaling web,” with many components and nodal points of control (4). One protein that has received recent attention and functions at such a convergent signaling nodal point, is PTEN.

The human tumor suppressor gene PTEN/MMAC1/TEP1 (PTEN, phosphatase and tensin homologue on chromosome ten; MMAC1, mutated in multiple advanced cancers-1; TEP1, TGF-β-regulated, epithelial cell-enriched phosphatase) is either deleted or inactivated in a high percentage of breast, endometrial, brain, and prostate cancers (5–7). PTEN is also linked to two dominantly inherited disorders, Cowden disease and Bannayan-Zonana syndrome, which lead to multiple defects, including excessive developmental growth of specific structures such as digits, formation of many benign outgrowths called hamartomas, and increased occurrence of cancer (8, 9). Tumor suppressor function has been confirmed by several gene ablation studies in mice, which show that mice with only one functional copy of the gene are more likely to develop tumors of multiple origins, and that homozygous loss of PTEN is embryonic lethal (10–12).

PTEN is a dual-specificity phosphatase with homology to the focal adhesion-associated protein tensin (13). In vitro, PTEN can dephosphorylate acidic polypeptides, focal adhesion kinase (FAK), and the adaptor protein Shc. However, the major in vivo substrate for PTEN may be phosphatidylinositol 3,4,5-triphosphate (PIP3), because embryonic fibroblasts taken from PTEN null mouse strains have abnormally high levels of PIP3 and are resistant to apoptosis (12). These fibroblasts, as well as several breast cancer and glioblastoma-derived cell lines, show extremely high levels of activated Akt, a serine/threonine kinase that is regulated by PIP3 and phosphatidylinositol 3,4-biphosphate (14, 15). Akt is an important regulator of both cell survival and cell growth (16). In work spanning worms to mammals, PTEN has been defined genetically and biochemically to act as a negative regulator of Akt in opposition to the evolutionarily con-
served GFF-1/P13K/Akt signaling pathway (17–19).

Transcriptome and proteome analyses of general biological processes are beginning to yield a general picture of normal and altered cell states (20, 21). We have used a modified transcriptome approach to examine those transcripts that are actively translated during cardiac hypertrophy. In the course of this screen, PTEN emerged as a strong candidate. In this study, we demonstrate that PTEN is differentially expressed during cardiac hypertrophy, and show that altered expression can impact on cardiomyocyte viability. PTEN can also function as a critical regulator of cardiomyocyte hypertrophy and survival. A PTEN mutant, H123Y, exhibits dominant-negative activity in cardiomyocytes, leading to increased Akt activation and cardiomyocyte hypertrophy. To our knowledge this is the first demonstration of a true dominant negative activity for a PTEN mutant in cultured cardiomyocytes and should contribute toward understanding the potential consequences of human tumorigenic PTEN mutations in other cell types.

**EXPERIMENTAL PROCEDURES**

**Reagents**—All cell culture media were from Life Technologies, Inc. unless otherwise stated. Antibiotics, insulin-transferrin-selenite, bradykinin (Sigma-Aldrich), and resuspended in 1:1 medium consisting of DMEM/F-12, 15 mM HEPES, 5 mM fetal bovine serum, and 1 μg/ml gentamicin to allow adherence of non-cardiomyocytes. Cells remaining in the supernatant were pelleted and resuspended in 1:1 medium consisting of DMEM/F-12, 15 mM HEPES, 5 mM horse serum, 2.5 mM μg/ml insulin, 2.5 mM μg/ml transferrin, 2.5 ng/ml sodium selenite, 30 μg/ml bromodeoxyuridine, and 1 μg/ml gentamicin. Viable cardiomyocytes, as determined by trypan blue exclusion, were plated at 250 cells/mm² on ProNectin-F-coated plastic cultureware (4 μg/ml coating solution in 1× PBS; BIOSOURCE International) and grown for 12–18 h in 1:1 medium at 37°C in 5% CO₂.

For virus infection, cells were infected at a multiplicity of infection (m.o.i.) of 10 infectious units (ifu)/cell for 1 h in DMEM/F-12 at 37°C. Following infection, cells were incubated in 1:1 medium for 48 h, pelleted, and resuspended in DMEM/F-12 for the remainder of the culture period, usually 24–48 h. For immunofluorescence, cells were grown in 4-well-chambered Permanox slides (Nalg Nunc) coated with ProNectin-F. Primary antibodies were used at 1:100 dilution on cells fixed in 4% paraformaldehyde/1× PBS and permeabilized in 40 mM HEPES (pH 7.4), 50 mM Pipes (pH 6.9), 10 mM EGTA, 5 mM MgCl₂, and 0.1% Triton X-100.

Replication-deficient recombinant adenoviruses were made using the AdEasy system described by He et al. (1998). XhoI-XbaI restriction fragments from either pWTPTENN5TOPO or pH123YPTENN5TOPO, spanning the insert and tag, were ligated into Xhol-XbaI-digested pShuttleCMV. To create AdLacZ, a NotI-NotI restriction fragment from pCMVβ (22) was ligated to NotI-digested pShuttleCMV. Recombination into the pAdEasy-1 viral backbone was accomplished in bacteria as described previously (23). Recombinant pAdEasy plasmids containing CMV-cDNA inserts were purified over Qiagen columns and 4 μg of PacI-digested DNA was used to transfect HEK293 cells by LipofectAMINE (Life Technologies). Cells were seeded at 2 × 10⁶ cells per 25-cm² flask 24 h prior to transfection. Lysis of transfected cells indicating adenoviral growth occurred by 4 days. Following amplification, recombinant adenovirus-containing supernatants were harvested from 10 75-cm² flasks and purified by CsCl gradient centrifugation. Recovered virus was aliquoted and stored at −20°C in 5 ml Tris (pH 8.0), 50 μM NaCl, 0.05% bovine serum albumin, and 25% glycerol. Virus was titered by serial dilution infection of HEK293 cells and counting of plaques under 0.3% agarose/10% fetal bovine serum/1× Dulbecco's modified Eagle's medium overlay. Cardiomyocytes were plated on duplicate 100-mm plates seeded with 1.96 × 10⁶ cardiomyocytes were either mock infected or infected with AdlaxZ, AdWTPTEN, or AdH123YPTEN at an m.o.i. of 10 ifu/cell. For infection, cells were lysed in cold lysis buffer containing 20 μM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 10 mM DTT, 1 mM PMSF, 20 μM sodium orthovanadate, and a protease inhibitor mixture (Complete protease inhibitor tablet, Roche Molecular Biochemicals). Protein concentration was determined by the Bio-Rad assay in triplicate, and the proteins were separated by discontinuous SDS-PAGE before transfer to polyvinylidene difluoride membrane for Western blot analysis. Rabbit polyclonal anti-PTEN antibody (Upstate Biotechnology Inc.) and monoclonal anti-PK-TAG (anti-V5) (Serotec), both titrated 1:2000 dilution, and monoclonal anti-GAPDH antibody (Chemicon, 1:5000 dilution) were used to probe the membranes. A PTEN mutant, H123Y, exhibits dominant-negative activity in cardiomyocytes, leading to increased Akt activation and cardiomyocyte hypertrophy. To our knowledge this is the first demonstration of a true dominant negative activity for a PTEN mutant in cultured cardiomyocytes and should contribute toward understanding the potential consequences of human tumorigenic PTEN mutations in other cell types.

**Cloning of PTEN cDNA**—Two hundred nanograms of total RNA from adult FVB/N mouse heart was used in a single-tube reverse transcriptase-polymerase chain reaction (Titan, Life Technologies) to amplify PTEN cDNA. The sense primer was 5'-AGGTCCTCCAGCATGA-CACCATC-3' (bases 937–960 of PTEN cDNA, GenBank accession number U92437), and the antisense primer was 5'-ACTTTTGTAATT-GCA ATT TAC TGT AAA GCT GGA AAG-3' (bases 2156–2190 of PTEN cDNA, GenBank accession number U11032). Site-directed mutagenesis to create the H123Y substitution was performed using the QuikChange kit (Stratagene) and the following complementary oligomers spanning bases 1300–1332: 5'-CAT GTT GCA GCA ATT TAC TGT AAA GCT GGA AAG-3' and 5'-CTT TGC AGC TTT ACA GTA TAT TGC AAC ATG-3'. Plasmids pWTPTENN5TOPO (WTPTEN) and pH123YPTENN5TOPO (H123YPTEN) were sequenced to verify integrity.

**Cell Surface Area Determination and [3H]Leu Assays**—Cardiomyocytes were seeded at 250 cells/mm² in 6-well culture dishes 12 h prior to viral infection. Twenty-four h following infection (or mock treatment) cardiomyocytes transduced with AdlaxZ, AdWTPTEN, or AdH123YPTEN were incubated for 8 h in leucine-free RPMI 1640 supplemented with 5
μCi/ml [3H]leucine. Cells were washed, and the proteins were precipitated with ice-cold 10% trichloroacetic acid then washed again with cold 95% ethanol, and the lysates were collected in 0.5 N NaOH for scintillation counting. Genomic DNA was extracted from duplicate samples in 10 mM Tris-HCl (pH 9.0), 0.1 M EDTA (pH 9.0), 20 μg/ml RNAase, and 0.5% SDS, incubated at 37°C for 1 h, then extracted in phenol/chloroform before ethanol precipitation. Recovered nucleic acid was quantified by absorbance at 260 nm and used to control for cell number. Relative cell surface area was calculated from digitized cell images taken from random fields of view using National Institutes of Health IMAGE software (version 1.62). At least 100 cells were counted per sample, and statistical analysis was carried out using Student’s t test.

Caspase-3 Assay—Four 100-mm plates were seeded with 1.96 × 10^6 cardiomyocytes for each sample. Cells were mock infected, infected with AdlacZ, AdWTPTEN, AdH123YPTEN, or treated with 0.5 μM staurosporine for 6 h prior to harvest. Trypsinized cells were scraped from the plates and counted. 1 × 10^6 cells from each sample (in duplicate) were lysed and used in a 100-μl reaction with 50 μM of the colorimetric DEVD-pNA substrate in 1× reaction buffer as per the manufacturer’s instructions (Caspase-3 Colorimetric Assay kit, CLONTECH). Five μM of the irreversible caspase-3 inhibitor, DEVD-fmk, was used as a control for specificity. The colorimetric reaction products were determined at 405 nm. All samples were done in triplicate, and data were analyzed by Student’s t test.

Akt Assay—Duplicate 100-mm plates seeded at 1.96 × 10^6 cardiac myocytes were either mock infected or infected with AdlacZ, AdWTPTEN, or AdH123YPTEN at an m.o.i. = 10 infectious units/cell. For a positive control mock infected cells were treated with 10 ng/ml of IGF-1 for 15 min prior to harvest. Where indicated AdWTPTEN-infected cells were also treated with 10 ng/ml IGF-1 in the same manner. For a negative control, cells were treated with 10 μM of the PI3K inhibitor LY294002 (Cell Signaling Technology) for 45 min prior to and during IGF-1 treatment. Cells were harvested 48 h after infection in 1 ml of 1× lysis buffer (20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM NaVO_4, 0.5 μM leupeptin, and 1 mM PMSF). Two hundred microliters of lysisate was then used for immunoprecipitation of Akt using 20 μl of immobilized anti-Akt antibody slurry (Akt kinase assay kit, Cell Signaling Technology). Pellets were washed twice in lysis buffer, then twice in kinase buffer (25 mM Tris (pH 7.5), 5 mM β-glycerophosphate, 2 mM DTT, 0.1 mM Na_3VO_4, 10 mM MgCl_2), before initiating the reaction with the addition of 200 μM ATP and 1 μg of GSK3-β peptide fusion peptide. Reactions were incubated at 30°C for 30 min, then terminated with 3× SDS sample buffer, and the reaction products were separated by discontinuous SDS-PAGE. The gels were transferred to polyvinylidene difluoride membrane, and the phosphorylated GSK-3-β fusion peptide was detected with anti-pGSK-3 antibody (1:1000 dilution, Cell Signaling Technology).

MTT Cell Survival Assay—Cardiomyocytes were seeded into 96-well plates at a density of 250 cells/mm^2 18 h prior to infection with adenovirus. Cells were mock infected or infected with AdlacZ, AdWTPTEN, or AdH123YPTEN at an m.o.i. of 10 if each for 1 h at 37°C, allowed to recover in DMEM/F12 medium for 1 h, then placed into 100 μl of DMEM/F12 without phenol red. At indicated time points, 10 μl of 5 mg/ml MTT was added to the wells, which were then incubated at 37°C for 4 h. Solubilization of the converted purple formazan dye was accomplished by placing cells in 100 μl of 0.01 N HCl/10% SDS and incubating overnight at 37°C. The reaction product was quantified by absorbance at 570 nm. All samples were done in triplicate, and data were analyzed by Student’s t test.

RESULTS

Detection of Transcripts That Shift to Polysomes during Hypertrophy—We initially sought to define genes that are differentially expressed during cardiac hypertrophy. Chronic isoproterenol (ISO) infusion in mice is a simple and well-characterized protocol that results in a characteristic 20–30% cardiac hypertrophy as measured by heart to body weight ratios (25). To increase the probability of finding genes that were altered at a lower level of protein expression, polysomes derived from mouse ventricles were gradient-purified and size-fractionated using velocity density centrifugation (Fig. 1A). Fractions were taken and the ventricular RNA isolated to select for the transcripts that were loaded onto polysomes in response to ISO (26). Four CLONTECH Atlas 1.2 macroarray filters were simultaneously hybridized to radiolabeled cDNA probes that were derived from either vehicle-treated (control) free or polysome-bound RNA, or from ISO-treated free or polysome-bound RNA (Fig. 1). For each array element, a numerical value for the shift to polysomes was calculated by taking the ratio of ISO bound/free signal divided by the ratio of vehicle bound/free signal. Signals were normalized to the median filter value to correct for differences in probe specific activities. A value of >1 indicates an increase for that transcript in the polysome fraction due to chronic ISO infusion. A typical block of array elements from the four filters (Fig. 1B) shows that the tumor suppressor PTEN (arrow) and the collagen IX α-2 subunit (arrowhead) undergo a 3.3-fold and 6.8-fold shift to polysomes, respectively (Table I). Beta actin and GAPDH were invariant. Interestingly, although the candidate genes exhibited a wide range of polysome shifts (~2- to 90-fold) almost all of the candidates exhibited only modest changes in total RNA amounts between control and hypertrophic hearts (0.6- to 30-fold) (Fig. 2, A and B). The lone exception was the serine/threonine kinase pmk-1, which normally is most highly expressed in myeloid cells (27). The candidate genes cover a spectrum of cellular functions such as signaling, cell architecture, transcription, proteolysis, and phosphorylation (Table I).

Increased PTEN Expression Causes Cardiomyocyte Apoptosis—Apparent transcript loading into heavy polysome complexes could be the result of either a block to translation elongation and hence decreased protein expression, or an increase in translation initiation and a resultant rise in protein production. One of the candidates, PTEN, has been linked to growth retardation of many cell types. Therefore, we hypothesized that it may play a similar role in the heart. Although the data in Fig. 2 showed that a number of transcripts exhibited a more robust response to isoproterenol stimulation, we undertook a detailed examination of PTEN to obtain a better understanding of the screening’s general value, and because of the involvement of PTEN in cell cycling in other systems. Quantitative
PTEN and Cardiac Hypertrophy

TABLE I
Candidate genes

| No. | Gene product | Function | ΔRNA | ΔPolysome |
|-----|--------------|----------|------|-----------|
| 1   | IL-1 receptor | Cell signaling | 0.8 | 3.1 |
| 2   | Galanin precursor | Cell signaling | 1.3 | 3.4 |
| 3   | HBEFG | Cell signaling | 0.9 | 8.8 |
| 4   | Inhibin-α | Cell signaling | 0.8 | 2.6 |
| 5   | Collagen 1Xα2 | Cytoskeleton | 1.2 | 6.8 |
| 6   | Skolemin | Cytoskeleton | 0.6 | 23.6 |
| 7   | Epas-1 | Transcription | 0.6 | 3.4 |
| 8   | mnt/rox | Transcription | 1.3 | 2.6 |
| 9   | NFAT 1α | Transcription | 1.1 | 2.8 |
| 10  | Paraxis | Transcription | 0.9 | 16.2 |
| 11  | Sp3 | Transcription | 1.8 | 2.1 |
| 12  | deltaEF-1 | Transcription | 0.9 | 2.3 |
| 13  | HPRT | DNA synthesis | 0.9 | 16.2 |
| 14  | LCAT | Transport | 0.8 | 8.8 |
| 15  | Calmodulin binding protein | Modulator | 0.6 | 36.3 |
| 16  | HCK | Modulator | 1.0 | 2.9 |
| 17  | p53IPK | Modulator | 1.2 | 5.3 |
| 18  | Annexin II light chain | Modulator | 0.8 | 13.4 |
| 19  | rab2 | Modulator | 1.7 | 3.0 |
| 20  | A-raf kinase | Kinase | 0.7 | 33.4 |
| 21  | c-Jun | Transcription | 0.8 | 3.8 |
| 22  | GPCR 27 | Receptor | 1.7 | 8.7 |
| 23  | pim-1 | Kinase | 5.3 | 3.2 |
| 24  | Cathepsin B1 | Protein Turnover | 0.9 | 2.4 |
| 25  | Cathepsin C | Protein Turnover | 1.3 | 9.4 |
| 26  | Cathepsin D | Protein Turnover | 0.7 | 16.2 |
| 27  | 5HT6c | Receptor | 0.9 | 4.9 |
| 28  | PTEN | Tumor suppressor | 1.1 | 3.3 |
| 29  | GPX3 | Stress | 1.2 | 3.3 |
|     | GAPDH | Metabolism | 1.2 | 0.9 |
|     | α-Actinin | Cytoskeleton | 1.0 | 0.8 |

FIG. 2. Polysome profiles change, although total RNA amounts remain stable. A, 29 candidate genes met the criteria outlined in Fig. 1 and under “Experimental Procedures.” The gene numbers correspond to Table I. B, total RNA changes were calculated by summing signals from all four array membranes. With the exception of the serine/threonine kinase, pim-1 (gene number 23), the candidate genes exhibited minimal RNA fluctuation.

Western blot analyses showed that PTEN protein expression is, in fact, induced in ISO-treated mouse hearts relative to vehicle-treated hearts (Fig. 3), in agreement with the polysome-derived data. Taken together with the shift of PTEN mRNA into the heavy polysome fractions during hypertrophy and the minimal change of total PTEN mRNA, this finding is consistent with regulation of PTEN expression by increased translational initiation. To address the consequences of altered PTEN expression in the heart, recombinant adenoviruses expressing wild-type PTEN or the catalytically inactive H123YPTEN mutant were examined by Western blot for PTEN expression. Only 4 μg of mouse brain extract was used as a positive control because of the very high expression levels of PTEN (note the weak GAPDH signal). Western blotting against GAPDH of the same membrane was used as an internal control for loading. B, the mean arbitrary values from four such experiments were calculated as follows: control, 6700 ± 2000; ISO 11,800 ± 3200. The asterisk indicates statistical significance.

FIG. 3. PTEN protein levels are increased in ISO hearts. A, 20 μg of total ventricular lysate from four untreated or ISO-infused hearts were examined by Western blot for PTEN expression. Only 4 μg of mouse brain extract was used as a positive control because of the very high expression levels of PTEN (note the weak GAPDH signal). Western blotting against GAPDH of the same membrane was used as an internal control for loading. B, the mean arbitrary values from four such experiments were calculated as follows: control, 6700 ± 2000; ISO 11,800 ± 3200. The asterisk indicates statistical significance.

Expression of H123YPTEN Leads to Cardiomyocyte Hyper trophy—Surprisingly, expression of the H123YPTEN mutant led to cardiomyocyte hypertrophy (Fig. 4C), with a well-ordered sarcomeric structure being conserved in the cardiomyocytes as shown by α-actinin staining (Fig. 4F). To determine if any molecular markers of hypertrophy were activated, we examined whether AdH123YPTEN-infected cells express atrial natriuretic factor (ANF), a readily identified marker of cardiomyocyte hypertrophy and stress, which accumulates in a perinuclear ring. Cells infected with either WTPTEN or H123YPTEN showed increases in perinuclear accumulations of ANF (Fig. 6), but the dominant negative mutant induced a much more robust response than the wild-type protein.

Other hallmarks of hypertrophy include increased cell volume and protein synthesis. Expression of H123YPTEN caused a >2.5-fold increase in cardiomyocyte cell surface area and a significant increase in [3H]leucine incorporation in the H123YPTEN-infected cells (Fig. 6, G and H). These data are consistent with the hypothesis that H123YPTEN can act in a dominant negative manner to enhance growth signaling in cardiomyocytes.

PTEN Alters the Activation of Akt in Cardiomyocytes—PTEN has been implicated genetically and biochemically as a negative regulator of insulin and insulin-like growth factors (12, 17–19, 31, 32). This is primarily accomplished by dephospho-
ordered sarcomeres; mutant results in cardiomyocyte hypertrophy as evidenced by the well-organized striations are apparent in contrast to the cells expressing WTPTEN. A, control cells were infected with lacZ-expressing virus (Ad-LacZ). Note the punctate cytoplasmic or membrane pattern and nuclear localization of endogenous PTEN. B, a sample field of the culture is shown 48 h after infection with PTEN. Overexpression of WTPTEN leads to a reduction in cell number. Cellular distribution of the protein is similar to endogenous expression. C, expression of the H123YPTEN mutant results in cardiomyocyte hypertrophy as evidenced by the well-ordered sarcomeres; F, when the cardiomyocytes are stained with α-actinin. D–F, the identity of the cardiomyocytes was confirmed by α-actinin antibody reactivity. In the H123Y-expressing cells, large well organized striations are apparent in contrast to the cells expressing WTPTEN. G, Western blot analyses. Western blots were carried out using anti-PTEN antibody. Cells were infected at an m.o.i. of 10 ifu/cell. Recombinant PTEN proteins contain a COOH-terminal V5 peptide tag and therefore migrate more slowly than endogenous PTEN. Mouse brain lysate was used as a positive control (Br). 1, no virus; 2, AdLacZ; 3, AdWTPTEN; 4, AdH123YPTEN. H, recombinant WTPTEN is a functional PI3P phosphatase, but H123YPTEN is not. Recombinant PTEN or H123YPTEN was immunoprecipitated using anti-V5 antibody on protein A/G beads for 4 h. The phosphatase assay was performed as described under “Experimental Procedures” with release of free phosphate measured colorimetrically. 1–4 correspond to the lanes in the Western blot (G).

ATP phosphorylation of 3′-phosphoinositides generated by PI3K stimulation. In this capacity, PTEN can decrease the activity of the important kinase, Akt, and indeed, PTEN null cells show an elevated basal activation of Akt (12, 32). Active Akt is phosphorylated on several key residues, and subsequently migrates from the cytosol to the plasma membrane and then to the nucleus (33, 34). We wished to determine the effects of expressing the wild-type and dominant negative forms of PTEN on the subcellular localization of Akt. As expected, expression of WTPTEN in cardiomyocytes blocks the nuclear accumulation of phosphorylated Akt (pAkt) that is seen under basal, non-stimulated conditions (compare Fig. 7A with 7B). Surprisingly, expression of the H123YPTEN mutant leads to an increase in nuclear accumulation of pAkt and apparent Akt activation (Fig. 7C). This was confirmed by an in vitro kinase assay to measure active Akt in cardiomyocytes (Fig. 7J). Compared with either untreated cells or to those that were infected with lacZ or WTPTEN-expressing virus, expression of H123YPTEN leads to an increase in Akt enzymatic activity in cardiomyocytes. Because H123YPTEN can cause cardiomyocyte hypertrophy, and this is accompanied by an increase in Akt activation, we conclude that H123YPTEN acts in a true dominant negative manner to effectively reduce the function of endogenous PTEN.

**PTEN Blocks Signaling by the Hypertrophic Agonist IGF-1—** Because PTEN is a downstream negative regulator of PI3K-mediated growth factor signaling, we next tested the ability of WTPTEN to block IGF-1 activation of Akt (Fig. 8). As expected, WTPTEN blocks nuclear accumulation of pAkt in response to 10 nm IGF-1. This effect also extends to estrogen, which activates Akt in vascular endothelial cells (35–37) and in cardiomyocytes.2 These observations are corroborated by an in vitro Akt kinase assay using lysates derived from cells overexpressing WTPTEN (Fig. 8G), confirming the ability of PTEN to block growth factor stimulation of the PI3K pathway in cardiomyocytes.

**DISCUSSION**

Transcriptome and proteome studies reveal a great deal about the global status of a tissue or cells. The data can sub-
expression seen in a perinuclear pattern characteristic of cardiomyocyte immunofluorescence using anti-V5 antibody against the V5-COOH-terminus fusion epitope placed in-frame on virally expressed PTEN proteins. C, expression of H123YPTEN leads to an increase in Akt expression seen in a perinuclear pattern characteristic of cardiomyocyte hypertrophy. B and E, cells that express WTPTEN and ANF, but to a lesser degree than that seen in H123YPTEN-expressing cells. G, H123YPTEN-expressing cardiomyocytes have increased surface area. Cell surface areas of uninfected or AdLacZ, AdWT PTEN-, or H123YPTEN-infected cells were measured by digital microscopy using National Institutes of Health IMAGE software. Values are relative to uninfected cells (*, p < 0.005 relative to uninfected cells); n ≥ 100. H, H123YPTEN causes increased \(^{3}\)H]leucine incorporation. Cells treated as in G were incubated in the presence of \(^{3}\)H]leucine for 6 h before lysis. Duplicate plates were processed in parallel for determination of DNA content as an internal control. All assays were done in triplicate, and the values reported are relative to uninfected cells. *p < 0.05 relative to uninfected control cells.

sequently be used to narrow an imposing (and growing) list of potentially interesting molecules to those that may be biologically active under a given set of conditions. Several of the genes obtained in our screen are associated with cardiac hypertrophy, including those encoding HB-EGF (38), c-Jun (39), annexin II (40), cathepsin B (41), and Sp3 (42). Interestingly, annexin II can complex with the protease cathepsin B on outer cell membranes to effect extracellular matrix remodeling (43), a process that occurs during cardiac hypertrophy. Annexin II can also bundle the actin filaments, modulating the cytoskeletal architecture (44), a process regulated by members of the multigenic Ca\(^{2+}\)-binding S100 proteins such as calcyclin (S100A6) in association with the calcyclin binding protein obtained in this screen (Table I (45, 46)). Two genes encoding the interleukin-1 receptor and lecithin:cholesterol acetyltransferase (LCAT), which were identified in the screen (Table I), are also induced by isoproterenol in other cell types (47, 48). The transcription factor Epas-1 (Table I) is normally associated with hypoxia and can induce the expression of enzymes involved in glycolysis (49). Epas-1 may therefore play a role in the transition from fatty acid oxidation to glucose metabolism observed in hypertrophic myocardium (50).

In preliminary Western blot experiments, about half of the proteins that were identified by the polysome-based screen increase in expression (eg Sp3, c-Jun, and HB-EGF), some proteins decrease in expression (cathepsin D), whereas others do not change (A-raf). Therefore, screening for altered gene expression during hypertrophy by polysome fractionation appears to be a reasonable protocol for whole tissue samples. Its advantage with respect to fractionation via staining of two-dimensional electrophoretic gels is that low abundance proteins such as transcription factors and kinases will be detected (51). An advantage over traditional arrays or microchips is that candidate genes are derived, based on polysome status, and thus are being actively translated (26).

Cardiac hypertrophy, a general adaptive response of the heart, can be stimulated in a number of ways, including the IGF-1/Pi3K/Akt pathway. The above data show that PTEN, a known antagonist to Pi3K signaling, can serve as a critical determinant of cardiomyocyte growth: It can block growth factor signaling, prevent hypertrophy, and, under certain circumstances, cause cell apoptosis. In tissue culture, expression of a dominant negative PTEN leads to increased cardiomyocyte viability and significant hypertrophy.

That expression of the H123YPTEN mutant caused the cultures of cardiomyocytes to hypertrophy was unexpected. Our original hypothesis was that the mutant would serve as a negative control. The H123Y mutation was originally isolated from a human endometrial tumor (13) and the mutation maps within the conserved “P-loop” that defines the catalytic core of dual specificity phosphatases (52). The mutant protein does not possess in vitro phosphatase activity (13) and, unlike the wild-

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\(^{3}\) G. Schwartzbauer and J. Robbins, unpublished observations.
cells were incubated with 10 nM IGF-1 for 15 min, 48 h post-infection, recombinant protein are shown by anti-V5 immunofluorescence. Arrows heads denote cells with a pronounced lack of nuclear pAkt.

Expression of PTEN blocked nuclear accumulation of pAkt in response to 10 nM IGF-1 (compare Fig. 7D to B). C and F, cells expressing recombinant protein are shown by anti-V5 immunofluorescence. Arrows heads denote cells with a pronounced lack of nuclear pAkt. G, expression of wild-type PTEN blocks Akt kinase activity. WTPTEN-infected cells were incubated with 10 nM IGF-1 for 15 min, 48 h post-infection, and compared with cells infected with AdLacZ in the presence (+) or absence (−) of IGF-1, the PISK inhibitor LY29004 was used as a negative control. Note the difference in Akt activation due to IGF-1 in the LacZ-infected cells versus the wild-type PTEN-expressing cells.

Wild-type PTEN blocks IGF-1 activation of Akt. A, B, D, E, phosphorylated Akt was detected by immunofluorescence as in Fig. 7. Expression of PTEN blocked nuclear accumulation of pAkt in response to 10 nM IGF-1 (compare A to B) or 10 nM 17β-estradiol (compare D to E), known activators of the PI3K pathway. Total Akt was distributed in a similar manner to pAkt. C and F, cells expressing recombinant protein are shown by anti-V5 immunofluorescence. Arrows heads denote cells with a pronounced lack of nuclear pAkt. G, expression of wild-type PTEN blocks Akt kinase activity. WTPTEN-infected cells were incubated with 10 nM IGF-1 for 15 min, 48 h post-infection, and compared with cells infected with AdLacZ in the presence (+) or absence (−) of IGF-1, the PISK inhibitor LY29004 was used as a negative control. Note the difference in Akt activation due to IGF-1 in the LacZ-infected cells versus the wild-type PTEN-expressing cells.

Figure 8. Wild-type PTEN blocks IGF-1 activation of Akt. A, B, D, E, phosphorylated Akt was detected by immunofluorescence as in Fig. 7. Expression of PTEN blocked nuclear accumulation of pAkt in response to 10 nM IGF-1 (compare A to B) or 10 nM 17β-estradiol (compare D to E), known activators of the PI3K pathway. Total Akt was distributed in a similar manner to pAkt. C and F, cells expressing recombinant protein are shown by anti-V5 immunofluorescence. Arrows heads denote cells with a pronounced lack of nuclear pAkt. G, expression of wild-type PTEN blocks Akt kinase activity. WTPTEN-infected cells were incubated with 10 nM IGF-1 for 15 min, 48 h post-infection, and compared with cells infected with AdLacZ in the presence (+) or absence (−) of IGF-1, the PISK inhibitor LY29004 was used as a negative control. Note the difference in Akt activation due to IGF-1 in the LacZ-infected cells versus the wild-type PTEN-expressing cells.

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