Tumor suppressor gene PTEN is highly mutated in a wide variety of human tumors. To identify unknown targets or signal transduction pathways that are regulated by PTEN, microarray analysis was performed to compare the gene expression profiles of Pten null mouse embryonic fibroblasts (MEFs) cell lines and their isogenic counterparts. Expression of a heparin binding growth factor, pleiotrophin (Ptn), was found to be up-regulated in Pten−/− MEFs as well as Pten null mammary tumors. Further experiments revealed that Ptn expression is regulated by the PTEN–PI3K–AKT pathway. Knocking down the expression of Ptn by small interfering RNA resulted in the reduction of Akt and GSK-3β phosphorylation and suppression of the growth and the tumorigenicity of Pten null MEFs. Our results suggest that PTN participates in tumorogenesis caused by PTEN loss and PTN may be a potential target for anticancer therapy, especially for those tumors with PTEN deficiencies.

PTEN (phosphatase and tensin homologue deleted on chromosome ten) is the first phosphatase identified as a tumor suppressor (1–3). Loss of function mutations or reduced expression of the PTEN gene are found at high frequencies in a wide variety of human tumors, including glioblastoma, as well as endometrial, prostate, colorectal, lung, and breast cancers. Experimental and clinical evidence demonstrate that PTEN deficiency is highly mutated in a wide variety of human tumors, including glioblastoma, as well as endometrial, prostate, colorectal, lung, and breast cancers. Experimental and clinical evidence demonstrate that PTEN loss and PTN may be a potential target for anticancer therapy, especially for those tumors with PTEN deficiencies.

PTEN acts primarily as a negative regulator of the phosphoinositide 3-kinase (PI3K) pathway by virtue of its lipid phosphatase activity (5–7). Loss of PTEN leads to an increase in the phosphatidylinositol 3-phosphate level, mimicking the effect of constitutive PI3K activation. Phosphatidylinositol 3-phosphate accumulation results in the activation of various protein kinases, including the PDK1 and PKB/AKT serine/threonine kinases. AKT is the central node in the PTEN-regulated pathway and activated AKT has been shown to promote cell cycle progression, cell growth, cell survival, cell migration, angiogenesis, protein synthesis, and glucose metabolism through phosphorylation of its substrates (8, 9).

Aside from acting as a negative regulator of the PI3K pathway, which depends on its lipid phosphatase activity, PTEN also functions independently of its lipid phosphatase activity. Raftopoulou et al. (10) showed that PTEN inhibits cell migration through its C2 domain and dephosphorylates itself depending on its protein phosphatase activity. Freeman et al. (11) demonstrated that PTEN physically associates with p53 in the nucleus, which in turn stabilizes p53 and affects p53 protein levels and transcription activity. Recently, Okumura (12) reports that the PTEN C-terminal domain physically interacts with the oncogenic MSP58 protein and suppresses cell transformation induced by MSP58 expression, independent of its catalytically active domain.

To further understand the biological functions of PTEN, we undertook an unbiased approach by comparing gene expression profiles of a Pten−/− mouse embryonic fibroblast (MEF) cell line with that of its isogenic counterpart (11, 13) using microarray technology. Among genes that are differentially expressed in these cell lines, we found that pleiotrophin (Ptn) was up-regulated in the Pten−/− MEFs as well as mammary tumor tissues of Pten conditional knock-out mice (14). Further experiments indicated that the expression of Ptn was regulated by the PI3K pathway, and Ptn overexpression contributes to tumorogenesis caused by PTEN loss.

**MATERIALS AND METHODS**

**Cell Culture and Generation of Immortalized Fibroblasts**—Fibroblasts were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, streptomycin, and penicillin, at 37 °C in 5% CO2. Immortalized wild-type (Pten+/−) and Pten−/− mouse embryonic fibroblast cells were generated independently according to the 3T9 protocol (13). The Pten−/− cell line was generated by infecting immortalized Pten+/−MEFs (Pten+/−) mouse embryonic fibroblasts with an adenovirus vector expressing Cre recombinase (11). Pten+/−-241 cell was clone-d from a tumor formed on nude mice by Pten+/− MEFS.

**Microarray Analysis**—Mouse cDNA microarrays containing 8,928 elements were printed by the UCLA Microarray Core Facility. Total RNA was isolated using TRIZol reagent (Invitrogen) according to the manufacturer’s instructions.

For probe labeling and microarray hybridization, a protocol published by Xiang et al. (15) was adopted with minor modifications. Briefly, cDNA probes that incorporate aminoallyl-dUTP (aa-dUTP; Sigma) were synthesized by reverse transcription using 10 μg of total RNA in a 30-μl reaction volume, containing oligo(dT) primer (0.5 μg/μl, 2 μl), RNase inhibitor (Promega, 5 units/μl, 1 μl), 1× first-strand buffer (Invitrogen), 0.5 mM dATP/dGTP/dCTP, 0.3 mM dTTP, 0.2 mM aa-dUTP, 10 mM dithiothreitol, and SuperScript II reverse transcriptase (Invitrogen, 1.9 μl). The reactions were incubated at 42 °C for 2 h and 37 °C for 1 h.

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terminated by addition of EDTA (0.5 mM, 10 μl). The RNA was hydrolyzed with NaOH (1 mM, 10 μl) and the solution was neutralized with HCl (1 mM, 10 μl), and then Microcon 30 concentrators (Millipore) were used to clean up the cDNA. Purified cDNA from Pten null and wild-type MEFs was labeled with either Cy5 or Cy3 nonreactive fluoros (Amer sham Biosciences), combined, and competitively hybridized to microarrays under a coverslip for 16 h at 65 °C. Microarray slides were scanned using the GenePix 4000A fluorescence scanner (Axon Instruments), variable photomultiplier tube voltage settings were used to obtain the maximal signal intensities with <1% probe saturation and balanced intensities between the two channels (ratio of medians ≈ 1). The experiment was performed in duplicate using independently isolated RNA samples.

Raw data files were generated and analyzed using the GenePix 3.0 microarray analysis software, then imported into Excel. Features for which R² values were below 0.4 and less than 50% of feature pixels that were 1 S.D. above background pixels in either channel were filtered out. Features with ratio of medians ≥2 were considered to be up-regulated and features with ratio of medians ≤0.5 were considered to be down-regulated.

*Extraction of RNA and Northern Blot Hybridization.*—RNA was isolated using TRIzol reagent (Invitrogen) according to the procedure provided by the manufacturer. Total RNA was separated on 1.0% agarose gels in the presence of formaldehyde, transferred to Hybond-N+ nylon membranes (Amer sham Biosciences), and hybridized in QuikHyb hybridization solution (Stratagene). Probes were labeled with [³²P]dCTP using the random priming kit (PrimeIt hybridization solution (Stratagene), and hybridized in QuikHyb and subcloned to the downstream H1 promoter in pSilencerTM 3.1-H1 (Ambion) using BamHI and HindIII. To test the knocking down abilities of the 3 siRNAs against Ptn, plasmids carrying Ptn-siRNA A, B, or C, or siRNA negative control (Ambion) were transiently transfected into 3T3 cells using Lipofectamine 2000 (Invitrogen), the cells were subjected to Northern hybridization using Ptn cDNA as probe. To generate cell lines stably expressing Ptn siRNA, 2 × 10⁶ PtenA3-A3 cells were transfected with plasmids carrying Ptn-siRNA A, B, or C or control siRNA, respectively, and cultured for 48 h. The cells were then selected with 200 μg/ml hygromycin for 2 weeks; the obtained clones were screened for Ptn knocking down by Northern hybridization.

*Cell Growth Curve.*—Cells were seeded into 24-well plates in triplicates at a density of 8 × 10³ per well, the number of live cells was determined by trypan blue staining and was scored daily for 7 days with a hemocytometer under an inverted microscope.

*Statistical Analysis.*—Microsoft Excel was used to analyze the data and plot curves. Analysis of variance was applied for multiple comparisons.

*Tumorigenicity in Nude Mice.*—Athymic female nude mice were each injected subcutaneously with 4 × 10⁶ PtenA3-A3 cells stably expressing Ptn siRNA B or control siRNA (n = 8 for each group). The mice were kept in pathogen-free environments and checked every 2 days. The dates, at which a palpable tumor first arose, and the weights of the tumors were recorded.

*Western Blot Analysis.*—Protein was extracted using RIPA buffer (1× phosphate-buffered saline, 1% Nonidet P-40, 0.1% sodium deoxycholate, 0.1% SDS and protease inhibitor mixture tablet (Roche)) and fractionated on SDS-polyacrylamide gels. Western blots were then probed with the antibodies against PTN (R&D Systems), and AKT, phospho-AKT (Thr-308), phospho-GSK-3β and β-actin, which were all purchased from Cell Signaling Technology. Horseradish peroxidase-conjugated secondary antibodies and LumiGLO™ reagent (Cell Signaling Technology) were used to detect specific binding, and signals were captured by x-ray film.

**RESULTS**

*Genes Differentially Expressed in Pten WT and Null MEF Cell Lines.*—To identify unknown targets or signal transduction pathways that are regulated by PTEN, we compared gene expression profiles of Pten WT and null mouse embryonic fibroblast cell lines (7, 13). Sixteen genes were found to be up-regulated with ratio of medians above 2.0 (Table 1), whereas 30 genes were found to be down-regulated with ratio of medians below 0.5 (Table 2), based on two independent microarray analysis. Eight of 10 differentially expressed genes could be confirmed by other means, such as Northern blot analysis, reverse transcriptase-PCR, or Western blot analysis (data not shown), indicating the high quality of our microarray analysis.

We further checked the expression statuses of these genes in another line of Ptn null MEFs, which is designated as PtenA3/A3. The PtenA3/A3 cell line was generated by transfecting immortalized Pten<sup>−/−</sup>MEF<sup>−/−</sup> mouse embryonic fibroblasts with an adenovirus vector expressing Cre recombinase. Ptn, among several other genes (data not shown), was also up-regulated in PtenA3/A3 cells (Fig. 1A).

An interesting observation to be noted is that up-regulation of Ptn in Pten-null MEFs is cell density-dependent: the differences in Ptn level between WT and Pten<sup>−/−</sup> MEF cells are more significant when cells reach high density. As shown in Fig. 1B, there was no difference in Ptn expression level between WT and Pten<sup>−/−</sup> MEF cells when they are on log phase, but significant difference was observed when they reached confluence, and a more significant difference was observed 2 days after they reached confluence (Fig. 1B). Because PTN, a secreted growth factor, can promote cell proliferation, migration, and angiogenesis (17–
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The cDNA of Pten \(^{-/-}\) MEF cells was labeled with Cy5, and the cDNA of WT MEF was labeled with Cy3, the ratio of medians is Cy5/Cy3, the ratios of medians from two independent experiments are shown here. The expression statuses of the genes in bold had been confirmed by Northern hybridization.

### TABLE 1
Genes that are up-regulated in Pten \(^{-/-}\) MEF cells

| Gene symbol | Description | Unigene No. | Ratio of medians |
|-------------|-------------|-------------|------------------|
| Cxcl12       | Chemokine (C-X-C motif) ligand 12 | Mm.302231 | 10.629 11.256 |
| Ccdn2       | Cyclin D2 | Mm.333406 | 4.892 4.088 |
| Trim10 (Rn9) | Tripartite motif protein 10 | Mm.299155 | 4.499 3.818 |
| Fln1        | Fibronectin 1 | Mm.193099 | 4.264 3.495 |
| Templ3      | Tissue inhibitor of metalloproteinase 3 | Mm.4871 | 3.93 3.263 |
| Slp1        | Secretory leucocyte protease inhibitor | Mm.375583 | 3.797 1.839 |
| Zip3611 (Bfr1) | Zinc finger protein 36, C3H type-like 1 | Mm.235132 | 3.458 2.576 |
| Bicc1       | Bic1: Bicaudal C homolog 1 (Drosophila) | Mm.268684 | 2.979 2.713 |
| Ptn         | Ptn | Mm.279690 | 2.966 3.345 |
| Sbn1p1      | Retinol-binding protein 1, cellular | Mm.277941 | 2.454 2.438 |
| Serpine2    | Serine (or cysteine) protease inhibitor, clade E, member 2 | Mm.3093 | 2.358 2.226 |
| Seppl       | Selenoprotein P, plasma, 1 | Mm.22699 | 2.293 2.48 |
| Slc39a10    | Solute carrier family 39 (zinc transporter), member 10 | Mm.370216 | 2.161 2.124 |
| Scarb2      | Scavenger receptor class B, member 2 | Mm.273379 | 2.148 2.04 |

**PTEN Expression Is Regulated by PI3K/AKT Pathways**—We then determined which pathway was involved in Ptn transcription regulation using various pathway-specific inhibitors. As shown in Fig. 3A, Ptn expression was significantly down-regulated by the PI3K inhibitor LY294002 at a concentration of 30 \(\mu M\) (Fig. 3A, third lane; quantification shown on the right panel). On the other hand, no significant changes of Ptn expression were seen when the mitogen-activated protein kinase kinase-specific inhibitor PD98059 was used, indicating that the expression of Ptn is regulated by PI3K pathways.

Chemical inhibitor treatment can only provide a rough picture on how Ptn is regulated. To further define the pathways that may be involved in Ptn regulation, we transfected various Pten constructs into...
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3T3 cells. As shown in Fig. 3B, transfection of wild-type Pten into 3T3 cells could suppress the expression of Ptn, whereas a non-functional, mutated form of Pten (C124S phosphatase-dead mutation) had no effect. Overexpression of wild-type and the activated form of Akt-1 also resulted in up-regulation of Ptn, whereas expression of a dominant-negative form of Akt-1 resulted in a down-regulation of Ptn expression (Fig. 3C), indicating that regulation of Ptn by PTEN is PI3K/AKT-dependent.

siRNA against Ptn Could Suppress the Malignant Phenotypes of Pten Null Cells—To assess the tumorigenicities of the Pten null cell lines in vivo, we injected Pten+/− MEFs into nude mice subcutaneously, and observed the mice for 4 months. Pten+/− MEFs could form tumors in nude mice with a latency of greater than 2.5 months. We then dissociated tumors and cultured the tumor cells in vitro. A clonally derived line, Pten+/−-241, was used for study as described below because of its shorter tumor forming latency.

To test if knocking down the expression of Ptn can reverse the malignant phenotypes of the Pten+/−-241 cells, we designed 3 siRNAs against the mouse Ptn gene. To test the knocking down abilities of the siRNAs against Ptn, 3T3 cells were transiently transfected with the plasmids expressing the Ptn siRNAs and subjected to Northern blot analysis. As shown in the left panel of Fig. 4A, Ptn-siRNA B and C had better knocking down abilities than Ptn-siRNA A. Plasmids containing Ptn-siRNA B or C were then stably transfected into Pten+/−-241 cells, 3 clones for Ptn-siRNA B and 2 clones for Ptn-siRNA C were picked, and Ptn expression levels were tested by Northern blots. The expression of Ptn in clone 2 of Ptn-siRNA B was almost totally knocked down, whereas clone 2 of Ptn-siRNA C had less reduction (Fig. 4A, right panel). We then measured growth properties of these two clones and compared the control siRNA-transfected cells (Fig. 4C). Consistent with expression levels of Ptn seen in Fig. 4B, the growth rate of clone 2 of Ptn-siRNA B was significantly decreased, whereas the growth rate of clone 2 of Ptn-siRNA C showed mild reduction, indicating that reduction of Ptn expression could inhibit the growth of Pten+/−-241 cells.

We then injected clone 2 of Ptn-siRNA B into nude mice. For the mice injected with Pten+/−-241 cells transfected with control siRNA, 5 of 8 developed tumors with a latency of 21 to 47 days; whereas in mice injected with clone 2 of Ptn-siRNA B, only one of eight had tumor with latency of more 90 days (Table 3). Taken together, these results indicate that knocking down the expression of Ptn could suppress the tumorigenicity of Pten null cells and overexpression of Ptn may indeed participate in tumorigenesis caused by PTEN loss.

Down-regulation of Akt Activity by Ptn siRNA—Previous studies have shown that PTN can induce the activation of the PI 3-kinase pathway in bovine epithelial lens cells (20), U87 glioblastoma cells (21), 3T3 cells (22), and human umbilical vein endothelial cells (23). So we examined the status of the PI 3-kinase pathway in the Pten+/−-241 cells transfected with Ptn-siRNA vectors. Consistent with previous studies, the phosphorylation levels of Akt and GSK-3β were significantly reduced in the Pten+/−-241 cells with Ptn knocked down (Fig. 4C), indicating that
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explain these discrepancies. First, PTEN could regulate different sets of genes in different cell types, tissues, or systems. Second, additional genetic alterations may be introduced during the process of model establishing, which is not directly related with PTEN expression. For example, alterations of p53 or p19 functions are usually associated with immortalizing MEFs (32). Obviously, shortcomings of the microarray technique itself, because of nonspecific binding caused by homologies between different genes, could contribute to the noise level of microarray analysis.

Due to the reasons mentioned above, the genes found to be differentially expressed in different systems is more likely to be the real candidates regulated by PTEN. We found that Ptn was up-regulated in two independent Pten null MEF cell lines as well as in Pten null mammary tumor tissues. We also demonstrated that the expression of Ptn is PI3K/AKT pathway-dependent.

PTN is a heparin binding secreted growth/differentiation factor that has diverse functions: being involved in cell activities of adhesion, migration, survival, growth, and differentiation (for reviews, see Refs. 17–19). Its growth and differentiation promoting activities may play a role in the precocious development observed in skin and mammary glands of Pten conditional knock-out mice, in which precocious hair follicle morphogenesis (33), excessive ductal branching, precocious lobulo-alveolar development, and pregnancy-associated milk-specific protein expression were observed (14).

Stable transfection of Ptn resulted in oncocogenic transformation of 3T3 cells and highly vascularized tumor formation in nude mice (34, 35). Ribozyme targeting of PTN suppresses the growth, angiogenesis, and metastasis of melanoma (36, 37) and pancreatic cancer cells (38), whereas overexpression of PTN has been observed in a variety of cancers (17–19). Because PTN is a secreted protein and can be detected in serum by enzyme-linked immunosorbent assay (39), PTN might be a tumor marker and has diagnostic value. Indeed, elevated serum PTN levels were found in patients with pancreatic cancer (39, 40), colon cancer (39), testicular cancer (41), lung cancer (42), and astrocytomas (43). Based on our observation PTN loss led to overexpression of PTN in vitro in cell culture systems and in vivo in the mammary tumors, and PTN plays a critical positive feedback role in controlling AKT activity. Further studies are worthwhile to determine whether PTN can be used as a predictive marker of the PTEN/PI3K/AKT pathway activation or expression status of Pten in animal models and clinical samples.

PTEN regulates the expression of the gene through various transcription factors, including hypoxia-inducible factor-1 (44), forkhead transcription factors (45), tumor suppressor p53 (11), NFκB (46), and β-catenin (47). Searching the transcription factor binding sites on the mouse Ptn promoter using the on-line tool Mat Inspector revealed that there are forkhead transcription factor FOXF2 and β-catenin/TCF/LEF-1 binding sites on the Ptn promoter. Whether or not they are involved in the up-regulation of Ptn in the Pten null MEFs and tumors need further investigation. Of note, the difference of PTN expression between Pten null cells and control is marginal when cells were cultured in low densities, whereas there was a significant difference in Ptn expression when cells reached high density, suggesting other signaling pathways sensitive to cell densities, or insensitive to contact inhibition, may be critical for the expression of Ptn.

In this paper we also showed knocking down the expression of Ptn could suppress the growth and tumorigenicity of PtenΔΔ-241 cells. Knocking down the expression of Ptn also resulted in the reduction of phosphorylation of Akt and GSK-3, suggesting that PTN played an important role in maintaining the activation of the PI 3-kinase pathway in PtenΔΔ-241 cells. PTN has been suggested to be a potential new...
target for the treatment or/diagnosis of several types of cancer (48). Gene therapy approaches of targeting PTN in established mouse tumor models using ribozymes (36, 37, 49–51) or antisense oligonucleotides (52) have been reported. Deficiency of PTEN has been linked with resistance to chemotherapeutic drugs, such as trastuzumab (Herceptin) (53), our data suggests that PTN-targeting therapies might be one of the candidates to overcome this resistance.

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REFERENCES

1. Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S. I., Puc, J., Miliaresis, C., Lesche, R., Martinez-Diaz, H., Rozengurt, N., Cardiff, R. D., Liu, X., and Wu, H. (2000) Science 287, 738–742
2. Zindy, F., Eischen, C. M., Randle, D. H., Kamijo, T., Cleveland, J. L., Sherr, C. J., and Slingerland, J. M. (2004) Cancer Cell 14, 401–404
3. Xiang, C. C., Kohl, O. A., Chen, M., Inman, J. M., Phan, Q. N., Chen, Y., and Brownstein, M. J. (2002) Nat. Biotechnol. 20, 738–742
4. Kohn, A. D., Summers, S. A., Birnbaum, M. I., and Roth, R. A. (1996) J. Biol. Chem. 271, 31372–31378
5. Kadomatsu, K., and Muramatsu, T. (2000) Cancer Lett. 147, 127–134
6. Muramatsu, T. (2002) Curr. Cancer Drug Targets 3, 339–346
7. Persad, S., Troussard, A. A., McPhee, T. R., Mulholland, D. J., and Dedhar, S. (2001) J. Biol. Chem. 276, 21358–21363
8. Papadimitriou, E., Polakis, A., Hatziapostolou, M., Partophysou, A., Polytarchou, C., and Mikeli, C. (2004) Curr. Cancer Drug Targets 4, 471–482
9. Czubayko, F., Riegel, A. T., and Wellstein, A. (1994) J. Biol. Chem. 269, 21358–21363
10. Czubayko, F., Schulte, A. M., Berchem, G. J., and Wellstein, A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14753–14758
11. Weber, D., Klomp, H. J., Czubayko, F., Wellstein, A., and Juhl, H. (2000) Cancer Res. 60, 5284–5288
12. Souttou, B., Juhl, H., Hackenbruck, J., Rockseisen, M., Klomp, H. J., Raulais, D., Vigny, M., and Wellstein, A. (1998) J. Natl. Cancer Inst. 90, 1468–1473
13. Klomp, H. J., Zerial, O., Flachmann, S., Wellstein, A., and Juhl, H. (2002) Clin. Cancer Res. 8, 823–827
14. Aigner, A., Brachmann, P., Beyer, J., Rager, J., Raulais, D., Vigny, M., Neubauer, A., Heidenreich, A., Weinreich, A., Czubayko, F., and Zugmaier, G. (2003) Oncol. Lett. 14, 1525–1529
15. Rager, J., List, B., Knobbe, C., Souttou, B., Raulais, D., Zeiler, T., Wellstein, A., Aigner, A., Neubauer, A., and Zugmaier, G. (2002) Br. J. Cancer 86, 858–863
16. Ulbricht, U., Brockmann, M. A., Aigner, A., Eckerich, C., Muller, S., Fillbrandt, R., Westphal, M., and Lamszus, K. (2003) J. Neuropathol. Exp. Neurol. 62, 1255–1275
17. Zhang, H., Chiles, K., Feldser, D., Laugher, E., Hanrahan, C., Georgesm, M. M., Simons, J. W., and Senzena, G. L. (2000) Cancer Res. 60, 1541–1545
18. Nakamura, N., Ramassamy, S., Vasquez, F., Signoretto, S., Loda, M., and Sellers, W. R. (2000) Mol. Cell. Biol. 20, 8869–8892
19. Agarwal, A., Das, K., Lerner, N., Sathe, S., Gieck, M., Casey, G., and Sizemore, N. (2005) Oncogene 24, 1021–1031
20. Persad, S., Troussard, A. A., McPhee, T. R., Mulholland, D. J., and Dedhar, S. (2001) J. Biol. Chem. 276, 1161–1174
21. Papadimitriou, E., Polakis, A., Hatziapostolou, M., Partophysou, A., Polytarchou, C., and Mikeli, C. (2004) Curr. Cancer Drug Targets 4, 471–482
22. Czubayko, F., Schulte, A. M., Missner, S. C., Hsieh, S. S., Colley, K. J., and Wellstein, A. (1995) Breast Cancer Res. Treat. 36, 157–168
23. Schulte, A. M., Lai, S., Kurtz, A., Czubayko, F., Riegel, A. T., and Wellstein, A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14753–14758
24. Malerczyk, C., Schulte, A. M., Czubayko, F., Bellon, L., Macejak, D., Riegel, A. T., and Wellstein, A. (2002) J. Biol. Chem. 277, 21358–21363