Pten signaling in gliomas[1,2]

Christiane B. Knobbe, Adrian Merlo, and Guido Reifenberger[3]

Department of Neuropathology, Heinrich-Heine-University, Düsseldorf, Germany (C.B.K., G.R.); and Neurosurgery/Brain Tumor Research, University Hospitals, Basel, Switzerland (A.M.)

In 1997, the *PTEN* gene (phosphatase and tensin homolog deleted on chromosome 10) was identified as a tumor suppressor gene on the long arm of chromosome 10. Since then, important progress has been made with respect to the understanding of the role of the Pten protein in the normal development of the brain as well as in the molecular pathogenesis of human gliomas. This review summarizes the current state of the art concerning the involvement of aberrant Pten function in the development of different biologic features of malignant gliomas, such as loss of cell-cycle control and uncontrolled cell proliferation, escape from apoptosis, brain invasion, and aberrant neoangiogenesis. Most of the tumor-suppressive properties of Pten are dependent on its lipid phosphatase activity, which inhibits the phosphatidylinositol-3′-kinase (PI3K)/Akt signaling pathway through dephosphorylation of phosphatidylinositol-(3,4,5)-tri phosphate. The additional function of Pten as a dual-specificity protein phosphatase may also play a role in glioma pathogenesis. Besides the wealth of data elucidating the functional roles of Pten, recent studies suggest a diagnostic significance of *PTEN* gene alterations as a molecular marker for poor prognosis in anaplastic astrocytomas and anaplastic oligodendrogliomas. Furthermore, the possibility of selective targeting of *PTEN* mutant tumor cells by specific pharmacologic inhibitors of members of the Pten/PI3K/Akt pathway opens up new perspectives for a targeted molecular therapy of malignant gliomas. *Neuro-Oncology 4, 196–211, 2002 (Posted to Neuro-Oncology [serial online], Doc. 02-002, May 13, 2002. URL <neuro-oncology.mc.duke.edu>)*

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[3]Address correspondence and reprint requests to Guido Reifenberger, Department of Neuropathology, Heinrich-Heine-University, Moorenstrasse 5, D-40225 Düsseldorf, Germany.

[4]Abbreviations used are as follows: Akt, v-*akt* murine thymoma viral oncogene homolog 1; CDKN1B, cyclin-dependent kinase inhibitor 1-$_B$; EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; HIF1, hypoxia-inducible factor 1; Mdm2, mouse double-minute 2 homolog; MMP, matrix metalloprotease; mTOR, mammalian target of rapamycin; PDK, 3′-phosphatidylinositol-dependent kinase; PI3K, phosphatidylinositol-3′-kinase; PIP_3, phosphatidylinositol-(3,4,5)-triphosphate; PTEN, phosphatase and tensin homolog deleted on chromosome 10; VEGF, vascular endothelial growth factor; WHO, World Health Organization.

The *PTEN*[4] gene, also known as *MMAC1* or *TEP1*, was identified in 1997 as a tumor suppressor gene that is altered by somatic mutations in various types of sporadic tumors, including malignant gliomas (Li and Sun, 1997; Li et al., 1997; Steck et al., 1997). In addition, germline mutations in the *PTEN* gene were found in patients with Cowden's disease (Liaw et al., 1997) and several other rare cancer-hamartoma syndromes, including Bannayan-Riley-Ruvalcaba syndrome (also known as Bannayan-Zonana syndrome) (Marsh et al., 1997, 1999), a subset of juvenile polyposis syndrome (Olschwang et al., 1998), Lhermitte-Duclos disease (Liaw et al., 1997; Wiestler et al., 2000), as well as Proteus and Proteus-like syndromes (Zhou, X.-P., et al., 2001). Since 1997, *PTEN* has been the topic of more than 600 scientific papers. In addition to the establishment of *PTEN* mutation profiles for a large variety of sporadic tumors (Ali et al., 1999), significant progress has been achieved in understanding the function of Pten, which acts mainly as a lipid phosphatase but may also have protein phosphatase activity. Pten is involved in the regulation of many important cellular functions, for example, cell-cycle progression, cell migration and

spreading, and cell growth and apoptosis (for recent reviews see Di Cristofano and Pandolfi, 2000; Maehama et al., 2001; Simpson and Parson, 2001; Yamada and Araki, 2001). We will summarize this exciting and rapidly progressing research field with a particular focus on the role of aberrant Pten signaling in malignant gliomas.

PTEN Gene Discovery

Li et al. (1997) employed representational difference analysis in combination with mapping of cancer-associated homozygous deletions on 10q23 to identify a gene that they termed *PTEN* for "phosphatase and tensin homolog deleted on chromosome ten." The same gene was independently isolated by Steck et al. (1997), who fine-mapped homozygous deletions in glioma cell lines and isolated the gene by exon trapping from a bacterial artificial chromosome spanning the critical region. These authors designated the identified gene *MMAC1* for "mutated in multiple advanced cancers." Both Li et al. (1997) and Steck et al. (1997) reported on tumor-associated mutations in various types of malignant cancers, including glioblastomas as well as prostate, breast, and kidney carcinomas. Li and Sun (1997) searched the GenBank EST (expressed sequence tag) database [http://www.ncbi.nlm.nih.gov/dbEST/index.html] for sequences containing conserved motifs of the tyrosine phosphatase catalytic domain and thereby identified a partial cDNA sequence, which was then used to isolate the corresponding full-length gene named *TEP1* for "TGF$_\beta$ -regulated and epithelial cell–enriched phosphatase." This name referred to the high expression of the gene in the HaCaT keratinocyte cell line, which could be down regulated by treatment with TGF$_\beta$. Today, *PTEN* is the official gene name approved by the Human Genome Organization (HUGO) Nomenclature Committee and used in the Mendelian Inheritance in Man database (OMIM 601728; "Appendix A").

PTEN Gene Location, Structure, and Regulation

The human *PTEN* gene maps to the chromosomal band 10q23.3 (Li et al., 1997; Steck et al., 1997). *PTEN* consists of 9 coding exons that span approximately 100 kb of genomic distance (Fig. 1). Fig. 2 illustrates the relationship between the individual coding exons and the domain structure of Pten. The *PTEN* promoter region has recently been characterized (Stambolic et al., 2001). The transcription start site lies between nucleotides –951 and –925. A positive regulatory element that drives constitutive expression maps between nucleotides –1001 and –427. In addition, the promoter carries a p53-binding element (between nucleotides –1190 and –1157). Functional analyses demonstrated that *PTEN* transcription may be transactivated by wild-type p53 (Stambolic et al., 2001). Li and Sun (1997) reported that *PTEN* can be transcriptionally down regulated by TGF$_\beta$. Furthermore, transcription can be induced by binding of Egr1 (early growth response 1 transcription factor) to a specific binding site (GCGGCGGCG) at

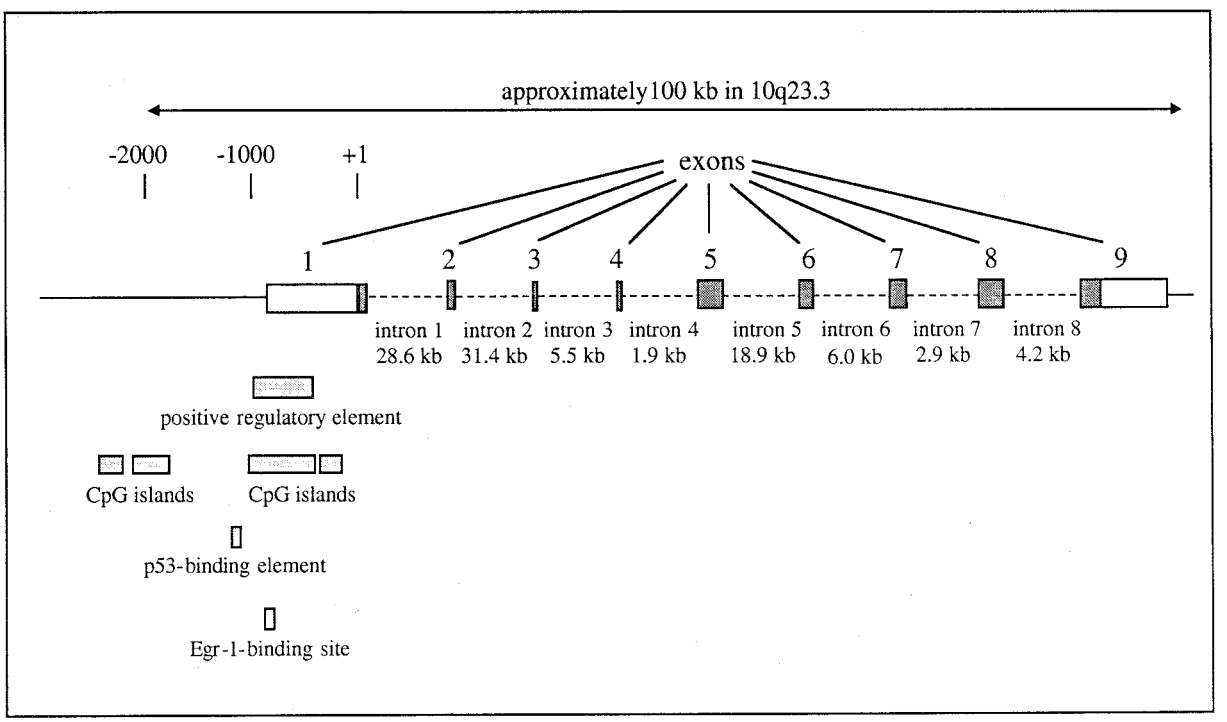

Fig. 1. Graphic representation of the genomic structure of *PTEN*. The gene consists of 9 exons (coding sequence indicated in gray color) and spans about 100 kb of genomic distance in 10q23.3. The promoter contains a positive regulatory element that is responsible for constitutive promoter activity. A p53-binding element maps between nucleotides (nt) –1190 and –1157. The Egr1 binding site locates between nt –947 and –939. The promoter is rich in CpG dinucleotides, with CpG islands being located between nt –2420 and –2211, nt –2113 and –1775, nt –1018 and –389, and nt –372 and –155.

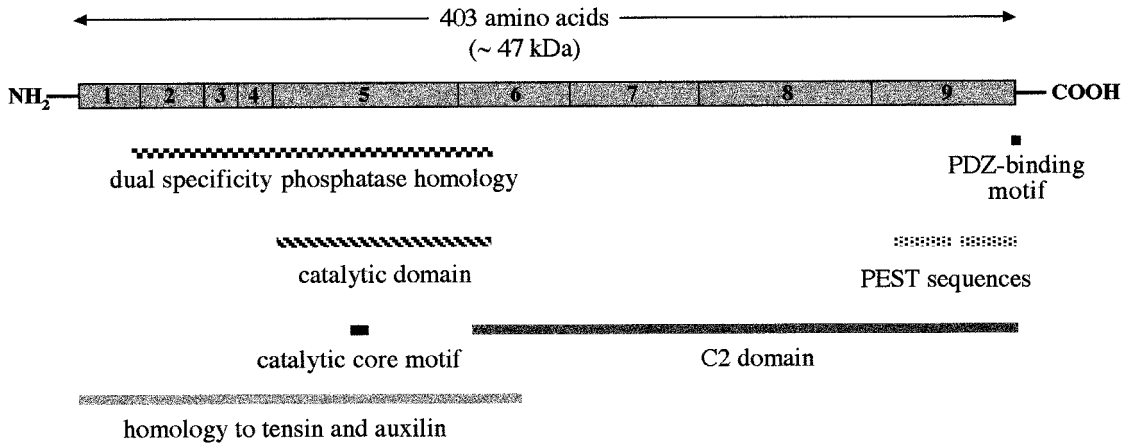

Fig. 2. Graphic representation of the Pten protein structure. The NH_2-terminal half of Pten shows homology to tensin and auxilin. It additionally carries the dual specificity phosphatase homology domain between amino acids (aa) 24 and 179. The catalytic domain maps between aa 87 and 177 with the catalytic core motif (GCXXGXXR) corresponding to aa 123-130. The COOH-terminal half of Pten contains a lipid-binding C2-domain between aa 234 and 403; proline, glutamic acid, serine, and threonine-rich sequences between aa 350 and 375 and aa 379 and 396; and a PDZ-binding motif corresponding to the COOH-terminal aa 401-403.

nucleotides –947 to –939 within the *PTEN* promoter (Virolle et al., 2001). The induction of *PTEN* transcription by Egr1 is important in the apoptotic response of cells to irradiation, which means that loss of either Pten or Egr1 function may contribute to radiotherapy resistance of cancer cells (Virolle et al., 2001).

The *PTEN* promoter is rich in CpG-dinucleotides, supporting a role of DNA methylation in transcriptional regulation of the gene. In line with this assumption, there is evidence from studies on different types of cancers that epigenetic silencing of *PTEN* caused by promoter hypermethylation may contribute to tumorigenesis (Salvesen et al., 2001; Whang et al., 1998). In gliomas, *PTEN* transcript levels decrease with increasing malignancy grade (Fults and Pedone, 2000; Sano et al., 1999). However, it remains to be investigated whether promoter hypermethylation is a mechanism of Pten inactivation in gliomas.

With the use of Northern blot analysis, 2 major *PTEN* transcripts of about 2 kb and 5 kb have been detected in various cell lines (Li and Sun, 1997; Li et al., 1997). Reverse transcriptase–polymerase chain reaction analyses identified 3 different alternative splice variants encoding proteins truncated at the COOH-terminal end (Schmidt et al., 1999; Sharrard and Maitland, 2000). The splice variant reported by Schmidt et al. (1999) lacks exon 6, which results in a frame shift in the coding sequence. This splice variant is expressed at low levels in addition to the normal transcript in various tissues including brain tissue. One of the splice variants reported by Sharrard and Maitland (2000) is generated by alternative splicing of the end of exon 5 to a splicing acceptor site within intron 5. This leads to an insertion of parts of intron 5 into the mRNA and an altered open reading frame that corresponds to a truncated protein lacking the amino acids encoded by exons 6 through 9. Another splice variant is generated by the inclusion of the 5′ end of intron 8 after exon 8, which results in a truncated protein that lacks the COOH-terminal amino acids encoded by exon 9 (Sharrard and Maitland, 2000). Expression of these splice variants was demonstrated in non-neoplastic cells as well as in various tumor cell lines (Sharrard and Maitland, 2000). All 3 splice variants encode proteins that lack important regulatory sequences at the COOH terminus, including regulatory phosphorylation sites; proline, glutamic acid, serine, and threonine-rich sequences; and the PDZ domain–binding motifs (see below and Fig. 2). In addition, the 2 variants truncated after exon 5 lack parts of the phosphatase catalytic domain. These splice products are, therefore, probably short-lived proteins without or with markedly reduced catalytic activity. However, this does not preclude a physiologic function, for example, as regulatory or modulating molecules (Schmidt et al., 1999; Sharrard and Maitland, 2000).

A *PTEN*-homologous gene named *PTEN2* has been identified in mice and humans (Wu et al., 2001). The Pten2 protein shows phospholipid phosphatase activity and is specifically expressed in the Golgi apparatus of secondary spermatocytes in the testis. *PTEN2* is not identical with the highly conserved processed *PTEN* pseudogene (*psiPTEN*), which has been mapped to 9p21 (Dahia et al., 1998) and has also been referred to as *PTEN2* (Forgacs et al., 1998). Fujii et al. (1999) detected *psiPTEN* transcripts (but no protein) in all investigated cells and tissues. There are conflicting data in the literature regarding whether *psiPTEN* is transcribed in human gliomas (Zhang et al., 2000) or not (Boström et al., 1998; Chiariello et al., 1999).

The *PTEN2* gene product is identical to the product of a gene referred to as *TPTE* (transmembrane phosphatase with tensin homology), which is specifically expressed in the testis, too (Chen et al., 1999; Guipponi et al., 2001). There appears to exist a family of closely related *TPTE* genes with loci mapping to chromosomes 13q and 21p, as well as 15, 22, and Y (Chen et al., 1999; Guipponi et al., 2001). The chromosome Y copy seems to be a pseudogene (Guipponi et al., 2001). S.M. Walker et al. (2001) also reported on the identification of a *TPTE*

gene, whose product is localized to the plasma membrane and shows no phosphoinositide-3-phosphatase activity. These authors found a further homologous gene that they termed *TPIP* (*TPTE* and *PTEN* homologous inositol lipid phosphatase), which gives rise to different splice variants. The $TPIP_A$ variant has phosphoinositide-3-phosphatase activity similar to that of Pten but localizes to the endoplasmic reticulum, whereas the TPIPB protein is cytosolic and lacks phosphatase activity (Walker, S.M., et al., 2001). It remains to be shown whether any of these homologs plays a role in tumor suppression.

Pten Protein Structure and Function

The full-length *PTEN* mRNA codes for a protein of 403 amino acids with a relative molecular mass of 47 kDa. The domain structure of Pten is shown in Fig. 2. The NH_2-terminal half of Pten contains the domain with homology to tensin and auxilin as well as the phosphatase domain. Determination of the Pten crystal structure showed that the phosphatase domain is structurally similar to protein phosphatases but has an enlarged active site, which is necessary for the accommodation of phosphoinositide substrates (Lee et al., 1999). The COOH terminus of Pten lacks catalytic activity but has important functions with respect to the regulation of protein stability, half-life, and functional activity (Georgescu et al., 1999, Lee et al., 1999). It carries a lipid-binding C2 domain that mediates interaction with the cell membrane and that is critical for productive positioning of the catalytic domain (Georgescu et al., 2000; Lee et al., 1999). Two proline, glutamic acid, serine, and threonine-rich sequences, which are involved in the regulation of Pten protein stability and degradation, are also located within the COOH terminus. A PDZ-binding motif maps to amino acids 401-403 and mediates the interaction of Pten with several PDZ domain–containing proteins, such as members of the membrane-associated guanylate kinase family (MAGI2, MAGI3, hDLG) and the microtubule-associated serine/threonine kinase MAST205 (Adey et al., 2000; Wu, X., et al., 2000; Wu, Y., et al., 2000). Binding to MAGI proteins enhances the ability of Pten to suppress Akt activation (Wu, X., et al., 2000; Wu, Y., et al., 2000). Finally, the COOH terminus is rich in phosphorylation sites. Phosphorylation of the COOH terminus by the protein kinase CK2 results in stabilization of Pten and prevents it from proteasome-mediated degradation (Torres and Pulido, 2001). In addition, Pten phosporylation may cause conformational changes that mask its PDZ binding domain and thereby reduce its ability to bind to PDZ domain–containing proteins (Vazquez et al., 2001).

With respect to the function of Pten, a major breakthrough was the finding that Pten shows phosphatase activity against phosphoinositide substrates (Haas-Kogan et al., 1998; Li et al., 1998; Maehama and Dixon, 1998; Stambolic et al., 1998; Wu et al., 1998). Pten dephosphorylates with high activity the 3′-OH position of the inositol ring of phosphatidylinositol phosphates, in particular of PIP_3, thereby acting as the counterpart of PI3K (Fig. 3). PIP_3 functions as a second messenger molecule that is important for the activation of protein kinase B/Akt (for reviews, see Vanhaesebroeck and Alessi, 2000; Scheid and Woodgett, 2001). PIP_3 facilitates the translocation of Akt to the plasma membrane. In addition, PIP_3 activates PDK1, which in turn phosphorylates Akt on threonine 308 within the kinase domain. For maximal activation, Akt needs to be additionally phosphorylated on serine 473 in its COOH-terminal regulatory domain. The identity of the kinase that phosphorylates Akt on serine 473 (PDK2) is a matter of ongoing research. It has been suggested that PDK1 can interact with a fragment of PRK2 (protein kinase C–related kinase 2), which has been termed PIF (PDK1-interacting fragment). Binding of PIF to PDK1 enables the enzyme to phosphorylate both threonine 308 and serine 473 of Akt (Vanhaesebroeck and Alessi, 2000). Activated Akt in turn can phosphorylate a variety of substrates and thereby regulates important cellular processes, including cell-cycle progression, cell growth, cell survival, cell motility and adhesion, translation of mRNA into protein, glucose metabolism, and angiogenesis (Fig. 3).

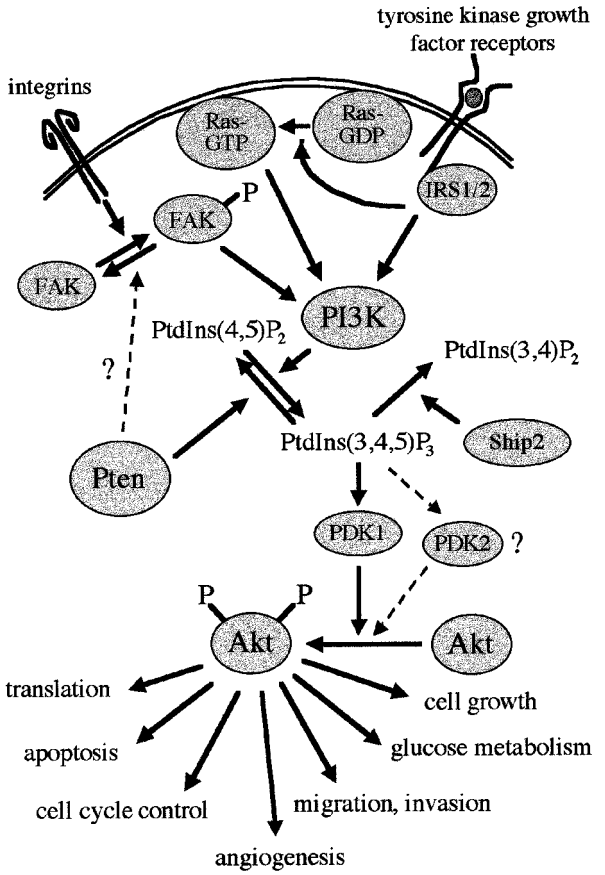

Fig. 3. Graphic representation of the Pten/PI3K/Akt signaling pathway. Pten functions in opposition to PI3K (phosphatidylinositol-3-kinase) by removing the phosphate from the 3′OH position of the phosphatidylinositol-(3,4,5)-triphosphate [PtdIns(3,4,5)P_3] signaling molecule. Activation of the pathway—for example, as a consequence of growth factor or integrin signaling—may result in pleiotropic cellular effects, as indicated in the figure. IRS1/2, insulin receptor substrates 1 and 2; PIF, PDK1-interacting fragment; Ship2, Src homology 2 domain containing inositol-5-phosphatase 2. Question marks indicate the open issues concerning the protein phosphatase activity of Pten against FAK and the identity of PDK2.

Pten may influence the activity of several cellular signaling pathways other than the PI3K/Akt pathway. For example, Pten may inhibit the integrin- and growth factor–mediated activation of the MAPK (mitogen-activated protein kinase) pathway, a capacity that seems to involve the phosphatase activity of Pten against protein substrates, such as the adaptor protein Shc (Src homology domain-containing transforming protein) (Gu et al., 1998, 1999) and IRS1 (insulin receptor substrate 1) (Weng et al., 2001a). In support of a role of Pten in the regulation of the MAPK pathway, Suzuki et al. (2001) reported that inactivation of Pten in T lymphocytes resulted in increased phosphorylation not only of Akt but also of Erk (extracellular signal–regulated kinase). The lipid phosphatase activity of Pten may also contribute to the inhibition of Ras and MAPK pathway activation by EGF (Yart et al., 2001). H-Ras–induced transformation and anchorage-independent growth of NIH3T3 cells could be blocked by Pten, an activity that was again dependent on the inhibition by Pten of the PI3K/Akt cascade (Tolkacheva and Chan, 2000). Furthermore, Pten can regulate the activity of the Wnt signaling pathway by inhibiting nuclear accumulation of β-catenin and β-catenin/TCF (T cell factor)-mediated transcriptional activation (Persad et al., 2001). By virtue of its inhibitory effects on Akt and integrin-linked kinase, wild-type Pten induces β-catenin phosphorylation by Gsk3 (glycogen synthase kinase 3), followed by enhanced β-catenin degradation and, consequently, down regulation of β-catenin/TCF target genes, such as the cyclin D1 gene *CCND1* (Persad et al., 2001). Finally, p53-dependent signaling may be modulated by the Pten/PI3K/Akt pathway through the phosphorylation of Mdm2 at serines 166 and 186 by activated Akt (Zhou, B.P., et al., 2001a). Phosphorylation of Mdm2 enhances its nuclear localization, inhibits its interaction with $p19^{ARF}$, and increases p53 degradation (Zhou, B.P., et al., 2001a). In line with these data, Pten has been shown to protect p53 from Mdm2-mediated degradation (Mayo et al., 2002). Because p53 induces *PTEN* transcription and Pten protects p53 from degradation, both proteins cooperate in a positive feedback loop to control the cellular response to stress, DNA damage, and cancer (Mayo et al., 2002). Taken together, these data reveal a network of complex interactions between different signaling pathways, and Pten is an important mediator of these interactions.

Specific Functions of Pten Related to Tumor Suppression

Numerous biochemical and cell biologic studies have implicated Pten in the regulation of a number of cellular processes that are important for tumorigenesis. In this section, we will briefly discuss the role of Pten in the regulation of cell-cycle progression, cell adhesion and migration, apoptosis, and angiogenesis.

Regulation of Cell Cycle Progression

Several studies reported that re-expression of wild-type Pten in originally Pten-deficient glioblastoma cells suppresses cell growth in vitro as well as tumorigenicity in nude mice (Cheney et al., 1998; Furnari et al., 1997, 1998; Li and Sun, 1998). The growth-suppressive activity of Pten is due to a cell-cycle arrest in the G_1 phase. Pten inhibits G_1 cell-cycle progression through inhibition of the PI3K/Akt pathway. Pten-induced G_1 arrest is pRB dependent and is associated with up regulation of the cyclin-dependent kinase inhibitor proteins $p27^{KIP1}$ and $p21^{Waf1}$ and with down regulation of D-type cyclins and cyclin A as well as with decreased Cdk2 (cyclin-dependent kinase 2) activity (Cheney et al., 1999; Gottschalk et al., 2001; Paramio et al., 1999; Weng et al., 2001b; Wu, R.C., et al., 2000). The induction of $p21^{Waf1}$ expression by Pten results from transcriptional up regulation of the *CDKN1A* gene (Wu, R.C., et al., 2000). In addition, direct phosphorylation of $p21^{WAF1}$ at threonine 145 by Akt has been shown to result in cytoplasmic localization of $p21^{WAF1}$, which suppresses $p21^{WAF1}$-dependent growth inhibition and promotes its anti-apoptotic activity through complex formation with Ask1 (apoptosis signal-regulating kinase 1) (Asada et al., 1999; Zhou, B.P., et al., 2001b). The increased expression of $p27^{KIP1}$ is due to Pten-induced transcriptional stimulation of *CDKN1B* via forkhead transcription factors (Medema et al., 2000; Nakamura et al., 2000) as well as inhibition of the ubiquitin-dependent degradation of $p27^{KIP1}$(Mamillapalli et al., 2001). A study by Gottschalk et al. (2001) indicated that $p27^{KIP1}$ is required for the Pten-induced G_1 arrest in glioblastoma cells, whereas p53 appears to be dispensable for this effect. Of note, the induction of $p27^{Kip1}$ represents an important determinant of growth at high cell density of glioblastoma cells in vitro (Fuse et al., 2000). Furthermore, immunohistochemical analyses of primary gliomas revealed that $p27^{Kip1}$ expression is inversely correlated with proliferative activity (as determined by MIB-1 staining) and malignancy grade (Fuse et al., 2000; Piva et al., 1997).

Regulation of Cell Adhesion, Migration, and Spreading

A distinctive feature of malignant gliomas is their invasive growth that leads to a diffuse infiltration of the adjacent brain tissue, thereby rendering the tumor surgically incurable. There are several lines of evidence implicating Pten in the regulation of cellular migration and invasion. Overexpression of Pten in NIH3T3 fibroblasts and U-87 MG glioma cells results in reduced cell spreading and migration on integrin substrates. Overexpression of Pten was associated with reduced levels of phosphorylation of FAK and $p130^{Cas}$, as well as a reduced number of focal adhesions (Tamura et al., 1998, 1999). There are 2 major ways in which Pten seems to inhibit FAK signaling. First, Pten may directly dephosphorylate and thereby inactivate FAK by means of its protein phosphatase activity (Tamura et al., 1998). However, more recent studies found that re-expression of Pten in U-87 MG cells did not reduce FAK phosphorylation (Jones et al., 2001a; Maier et al., 1999). Thus, the significance of Pten-mediated dephosphorylation of FAK in gliomas remains a controversial issue. Second, as activated FAK leads to activation of PI3K, Pten can interrupt this signaling by counteracting PI3K through dephosphorylation of PIP_3. The importance of FAK in tumorigenesis is

supported by the finding of *FAK* gene amplification and overexpression in several carcinoma cell lines (Agochiya et al., 1999). In glioblastomas, however, we did not detect any *FAK* gene amplification (Knobbe and Reifenberger, 2001), although FAK was found highly expressed at the protein level in astrocytomas and glioblastomas (Jones et al., 2001a), suggesting that FAK overexpression is differently regulated in malignant gliomas. In this study, a high level of FAK immunoreactivity was detected in most anaplastic astrocytomas and glioblastomas, as well as in low-grade diffuse astrocytomas, but was virtually absent in oligodendrogliomas of WHO grade II. In this context, the observation of increased FAK expression in a recurrent glioblastoma after radiotherapy as compared with the original tumor is intriguing and suggests selection of a more invasive phenotype by sublethal radiation therapy (Jones et al., 2001a). FAK is involved in the regulation of gene expression of anti-apoptotic genes such as *CIAP1* (*BIRC2*), *CIAP2* (*BIRC3*), *XIAP* (*BIRC4*), and *BCL2* because dominant-negative inactivation of FAK leads to reduced expression of these prosurvival factors (Jones et al., 2001a). Moreover, it has been shown that inhibition of FAK by dominant-negative FAT (focal adhesion targeting domain) not only attenuated EGFR signaling, but also enhanced EGFR degradation, thus inhibiting EGF-dependent migration (Jones et al., 2001b). Blocking FAK this way increased caspase-3 activity. The increase in apoptosis upon inhibition of FAK induced the aggregation of an NH_2-terminal FAK fragment normally present in the nucleus. Therefore, loss of FAK from the focal adhesions inhibits EGFR signaling at the cell membrane and might transmit a proapoptotic signal to an NH_2-terminal variant of FAK present in the nucleus (Jones et al., 2001b).

Kubiatowski et al. (2001) reported that specific inhibitors of PI3K (LY294002 and wortmannin) significantly reduced glioma cell invasiveness in vitro. This effect was accompanied by reduced enzymatic activity of the matrix metalloproteases MMP2 and MMP9. In line with these data, re-expression of wild-type Pten in the U-251 MG and U-87 MG glioma cell lines strongly decreased MMP2 mRNA levels and MMP2 enzymatic activity, and resulted in markedly reduced in vitro invasion (Koul et al., 2001). The recent report by Kotelevets et al. (2001) indicates that the lipid phosphatase activity of Pten is critical for both stabilizing intercellular junctions and reducing cell invasiveness. However, another study showed that invasion of glioma cells may also be inhibited by Pten independent from its phosphatase activity, suggesting that Pten can influence cellular migration and invasion through additional mechanisms (Maier et al., 1999).

Liliental et al. (2000) reported that immortalized fibroblasts from $Pten^{-/-}$ mice showed increased cell motility compared with wild-type control cells, a finding that could be normalized by reintroducing wild-type Pten but not lipid phosphatase–deficient Pten. The increased cell motility of $Pten^{-/-}$ fibroblasts was associated with strongly elevated Rac1-GTP and Cdc42-GTP levels but not with altered phosphorylation of FAK. These small GTPases are involved in regulating the actin cytoskeleton. Thus, in addition to interfering with integrin signaling, Pten may suppress cell motility by down-regulating Rac1-GTP and Cdc42-GTP.

Regulation of Apoptosis

Neoplastic cells often show reduced susceptibility to apoptotic stimuli. This phenomenon is also observed in malignant gliomas, which frequently lack sensitivity to radiation and/or chemotherapy. Pten plays an important role in maintaining the cellular susceptibility to apoptotic stimuli. *PTEN* gene transfer into U-87 MG glioma cells that lack wild-type *PTEN* renders the cells susceptible to apoptosis/anoikis (Davies et al., 1998; Tian et al., 1999). In addition, *PTEN* gene transfer sensitized glioma cells for apoptosis when irradiated or treated with FasL (Wick et al., 1999). The regulatory function of Pten on apoptosis is dependent on PI3K/Akt signaling. Pten wild-type cells overexpressing mutant (constitutively active) Akt are resistant to multiple apoptotic stimuli, as are Pten-mutant cells. Akt itself is a serine/threonine kinase that can phosphorylate several apoptosis-associated proteins, including the proapoptotic factor Bad (Bcl2 antagonist of cell death). Upon phosphorylation, Bad can no longer function as a proapoptotic molecule and dissociates from BclxL or Bcl2, leading to a relative increase in the level of anti-apoptotic proteins. Although the role of Bad in Akt-mediated inhibition of apoptosis is well characterized, it is likely that Pten/PI3K/Akt signaling may additionally influence apoptosis using other targets, such as glycogen synthase kinase 3, p70S6k, or I_κ B/NF_κ B (Maehama et al., 2001; Scheid and Woodgett, 2001).

Regulation of Angiogenesis

A histologic hallmark of glioblastoma is the presence of aberrant microvascular proliferation. Hypoxia as well as up regulation of certain growth factors, in particular VEGF and its receptors, have been shown to be of paramount importance for neoangiogenesis in glioblastomas. In fact, hypoxia stimulates the expression of HIF1, which in turn enhances the transcription of VEGF. Recent evidence suggests that not only hypoxia but also certain genetic alterations in glioma cells, such as *PTEN* mutation, *EGFR* amplification/rearrangement, and *PDGF* (platelet-derived growth factor) overexpression, may facilitate angiogenesis in glioblastomas and other tumors (Jiang et al., 2001; Maity et al., 2000; Wang et al., 1999; Wen et al., 2001; Zundel et al., 2000). Indirect evidence for a role of Pten in angiogenesis was provided by the observation that *PTEN*-mutant prostate carcinomas were histologically more densely vascularized than were *PTEN* wild-type prostate carcinomas (Giri and Ittmann, 1999). Zundel et al. (2000) reported that expression of wild-type Pten in a *PTEN*-deficient glioblastoma cell line decreased hypoxia- and IGF1 (insulin-like growth factor 1)-induced activation of Akt1. Wild-type Pten re-expression also reduced the expression of various hypoxia-inducible genes, including VEGF. In addition, wild-type Pten attenuated the hypoxia-mediated stabilization of $HIF1_\alpha$. The loss of Pten function may, therefore, facilitate $HIF1_\alpha$-induced gene expression and angiogenesis in glioblastomas. In line with these data, Jiang et al. (2001) reported that an inactivating *PTEN* mutation specifically increased the protein levels of $HIF1_\alpha$, but not of $HIF1_\beta$, in human cancer cell lines. Concomitantly, VEGF was transcriptionally down regu-

lated by expression of either a dominant-negative PI3K-construct or by wild-type Pten. Thus, Pten/PI3K/Akt signaling is important in the regulation of HIF1$_\alpha$ activity and VEGF expression. Wen et al. (2001) also showed that Pten can induce expression of thrombospondin 1, a potent antiangiogenic factor, and may thereby control tumor-induced angiogenesis. Other authors demonstrated that growth factors (for example, EGF and PDGF) and Ras may induce VEGF expression via the activation of the Pten/PI3K/Akt pathway (Blancher et al., 2001; Maity et al., 2000; Wang et al., 1999). Maity et al. (2001) reported that *PTEN* mutation can cooperate with EGFR activation to increase VEGF mRNA levels by transcriptionally up regulating the proximal VEGF promoter. Taken together, there is strong evidence that activation of the Pten/PI3K/Akt signaling pathway (through growth factor–receptor overexpression or *PTEN* mutation or aberrations in other pathway member genes) is important for the development of aberrant vascular proliferation in glioblastomas.

PTEN Mouse Models

The importance of Pten in embryonic development and tumor suppression has been substantiated by studies employing *PTEN* knock-out mice. Deletion of both *PTEN* alleles in mice ($PTEN^{-/-}$) resulted in early embryonic lethality, indicating that Pten is essential for embryonic development (Di Cristofano et al., 1998; Podsypanina et al., 1999; Suzuki et al., 1998). Targeted deletion of *PTEN* in nestin-positive neural stem/progenitor cells caused the formation of a morphologically abnormal, enlarged brain due to increased neural stem cell/progenitor cell proliferation, reduced apoptosis, and enlarged cell size (Groszer et al., 2001). Similarly, targeted deletion of *PTEN* in neuronal cells (Kwon et al., 2001) or glial cells (Backman et al., 2001) caused brain enlargement accompanied by seizures, as well as dysplastic changes in the cerebellum resembling Lhermitte-Duclos disease. *PTEN* heterozygous ($PTEN^{+/-}$) mice showed hyperplastic-dysplastic changes in the prostate, endometrium, skin, thyroid, and gastrointestinal tract (Di Cristofano et al., 1998; Podsypanina et al., 1999), most of which may also occur in human patients with heterozygous *PTEN* germline mutation. In addition, $PTEN^{+/-}$ mice are susceptible to the development of various types of tumors, including T-cell lymphomas, germ cell neoplasms, thyroid tumors, and colon tumors (Di Cristofano et al., 1998; Podsypanina et al., 1999; Suzuki et al., 1998). $PTEN^{+/-}$ mice also show aberrations of the immune system, including an impaired Fas-mediated apoptosis and the development of a lethal autoimmune disorder (Di Cristofano et al., 1999). A strikingly similar phenotype was observed in transgenic mice expressing an active form of PI3K in T lymphocytes (Borlado et al., 2000). These mice developed an infiltrating lymphoproliferative disorder as well as an autoimmune renal disease with an increased number of memory T cells and reduced apoptosis.

Di Cristofano et al. (2001) found that $PTEN^{+/-}$ mice with concomitant inactivation of one or both *CDKN1B* ($p27^{Kip1}$) alleles showed an accelerated spontaneous neoplastic transformation and an increased incidence of tumors of various histologic origins. Most notably, these mice invariably developed prostate cancer within the first 3 months of life. Cell proliferation, but not cell survival, was increased in the $PTEN^{+/-}/CDKN1B^{-/-}$ mice (Di Cristofano et al., 2001). Thus, there is an additive effect of combined Pten and $p27^{Kip1}$ deficiency with respect to increased cell proliferation.

Development of gliomas was not observed in $PTEN^{+/-}$ mice, a finding that is in line with the fact that Cowden's syndrome is not associated with an increased incidence of gliomas. However, a recent study experimentally confirmed the importance of Akt pathway activation for the development of human glioblastomas by using a human astrocyte model (Sonoda et al., 2001). The authors showed that human astrocytes engineered to express E6/E7 (for inactivation of the p53/pRb pathway), hTERT (human telomerase reverse transcriptase), and mutant H-Ras formed anaplastic astrocytomas when transplanted s.c. or i.c. in immunodeficient mice. Additional transfection of these cells with a constitutively active form of Akt resulted in the development of bigger transplant tumors with large necroses and neovascularization, which morphologically resembled the histologic picture of human glioblastoma. Another study on transgenic mice reported that neither activated Ras nor Akt alone was sufficient to induce glioblastoma formation. However, the combined expression of activated Ras and Akt induced malignant gliomas with features of human glioblastomas (Holland et al., 2000). Interestingly, malignant gliomas developed only after gene transfer to neural progenitors but not after transfer to differentiated astrocytes, indicating the importance of additional cellular factors for tumor susceptibility.

Expression of Pten in Normal Tissues

Using immunohistochemistry, Gimm et al. (2000a) investigated the pattern of Pten expression in human tissues during development. Strong Pten immunoreactivity was found mainly in tissues that are known to be affected in Cowden's syndrome and Bannayan-Riley-Ruvalcaba syndrome, such as skin, thyroid, and CNS tissues. In addition, Pten was found to be expressed at high levels in the developing peripheral and autonomic nervous system. In brain, Pten expression was high throughout embryonic and fetal development and was still detectable at a lower level in adults (Gimm et al., 2000a). Other authors detected Pten immunoreactivity in human fetal brain at 16, 23, and 27 weeks of gestation, but not in the adult brain (Fults and Pedone, 2000). In mouse brain, Pten is expressed not only in glial cells but also in neurons and may play a role in the regulation of neuronal differentiation, cell size, and apoptosis (Groszer et al., 2001; Kwon et al., 2001; Lachyankar et al., 2000). Immunofluorescence analysis revealed that Pten is localized mainly in the cytoplasm (Li and Sun, 1997). However, for several cell types—for example, follicular thyroid cells, keratinocytes and neurons—nuclear- and/or nuclear membrane–associated expression has also been reported (Gimm et al., 2000b; Lachyankar et al., 2000).

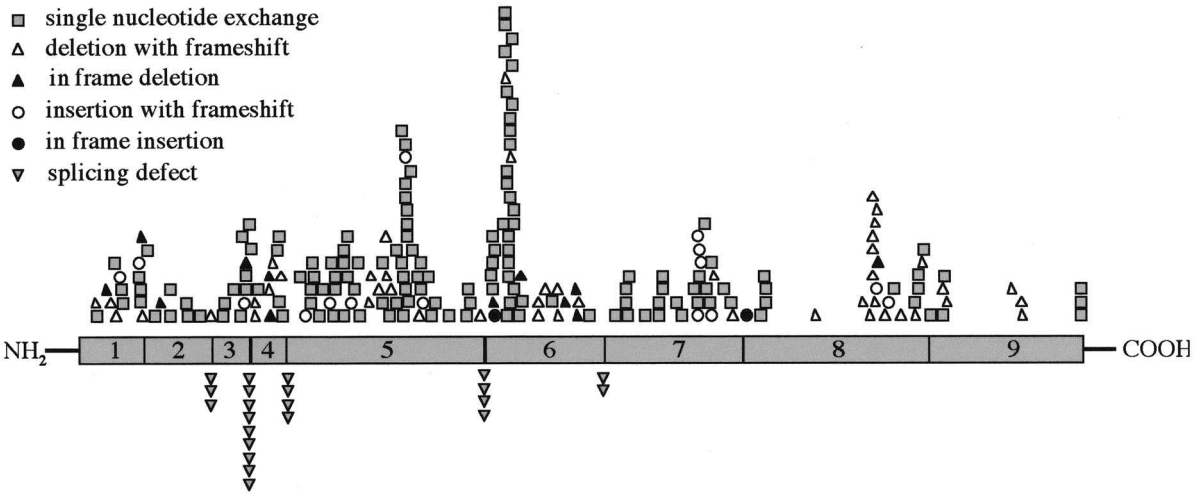

Fig. 4. Graphic representation of the distribution of 241 somatic *PTEN* mutations detected in human malignant gliomas. The mutations were compiled from the following references: Boström et al., 1998; Chiariello et al., 1998; Davies et al., 1999; Duerr et al., 1998; Georgescu et al., 1999; He et al., 2001; Kato et al., 2000; Knobbe and Reifenberger, 2001; Kraus et al., 2001; Lee et al., 1999; Liu et al., 1997; Peraud et al., 1999; Rasheed et al., 1997; Reis et al., 2000; Sasaki et al., 2001; Schmidt et al., 1999; Tohma et al., 1998; Tortosa et al., 2000; Walker, C., et al., 2001; Wang et al., 1997; Watanabe et al., 1998; Zhang et al., 2000; Zhou et al., 1999a. Note that the mutations are distributed along the entire gene. However, a clustering of mutations is seen within exon 5 and at the beginning of exon 6, i.e., the exons encoding the catalytic domain of Pten.

PTEN Gene Alterations in Gliomas

After the original studies on *PTEN* mutations in primary gliomas and glioma cell lines (Li et al., 1997; Steck et al., 1997), several other groups investigated larger series of different types of gliomas and other brain tumors for somatic *PTEN* alterations (for example, Boström et al., 1998; Duerr et al., 1998; Liu et al., 1997; Rasheed et al., 1997; Schmidt et al., 1999; Smith et al., 2001; Wang et al., 1997; Zhou et al., 1999a). As shown in Fig. 4, the mutations detected in gliomas are distributed along the entire gene. Mutation clusters are located within exon 5 and the 5′ part of exon 6, which encode major parts of the phosphatase domain. More than half of the mutations detected in gliomas result in the introduction of premature stop codons and the translation of truncated proteins. Missense mutations generally affect conserved residues where substitution leads to structurally altered proteins with impaired function. In addition to nonsense and missense mutations, several mutations resulting in aberrantly spliced transcripts have been detected. Homozygous *PTEN* deletions are present in about 5% to 10% of glioblastomas. The possible role of mutations or hypermethylation in the promoter region in gliomas needs to be addressed.

In gliomas, *PTEN* mutations are preferentially found in glioblastomas, with reported frequencies in unselected series ranging from 15% (Duerr et al., 1998) to 40% (Schmidt et al., 1999). The variable mutation frequencies between different studies are at least partly due to differences in the sensitivity of the applied techniques for mutation detection, for example, single-strand conformation polymorphism analysis (Duerr et al., 1998) versus denaturing gradient gel electrophoresis (Schmidt et al., 1999). However, it is also important to know that *PTEN* mutations are frequent (up to 40% of the cases) in primary (de novo) glioblastomas, giant cell glioblastomas, and gliosarcomas, but are rare (<10% of the cases) in secondary glioblastomas, i.e., glioblastomas that have progressed from pre-existing lower grade gliomas (Peraud et al., 1999; Reis et al., 2000; Tohma et al., 1998; Tortosa et al., 2000). *PTEN* gene alterations in glioblastomas do not correlate with either *EGFR* amplification or *TP53* mutation (Liu et al., 1997; Rasheed et al., 1997; Schmidt et al., 1999; Smith et al., 2001). *PTEN* aberrations are detectable in a low fraction (<10%) of anaplastic astrocytomas and anaplastic oligodendrogliomas (Sasaki et al., 2001; Smith et al., 2001), but are either rare or absent in WHO grade I and II astrocytic, oligodendroglial, and mixed gliomas; glioneuronal tumors; and low-grade and anaplastic ependymal tumors (Duerr et al., 1998; Ebert et al., 1999; Maier et al., 1998; Zhou et al., 1999a).

Several studies have investigated the possibility of germline *PTEN* mutations in cases of familial glioma (Malmer et al., 2001; Tachibana et al., 2000; Zhou et al., 1999a). However, no *PTEN* germline alterations were detected in glioma families.

Alterations of Other Pten/PI3K/Akt Signaling Pathway Members in Gliomas

Most human glioblastomas show high levels of activated Akt (Holland et al., 2000), whereas less than half of the cases carry *PTEN* mutations or homozygous deletions. It is possible that the actual percentage of glioblastomas with Pten aberrations may be higher because other mechanisms may cause down regulation or loss of expression of the protein. For example, Sano et al. (1999) reported that about two thirds of glioblastomas lack detectable Pten protein expression. However, recent data indicate that not only *PTEN* but also other genes of the Pten/

PI3K/Akt pathway may be altered in human tumors, including glioblastomas. For example, the *AKT2* gene may be amplified and overexpressed in ovarian and pancreatic carcinomas (Cheng et al., 1992, 1996). The *PIK3CA* gene at 3q26.3, which encodes the p110$_{\alpha}$ subunit of PI3K, is amplified in subsets of cervical and ovarian carcinomas (Shayesteh et al., 1999; Ma et al., 2000). A gain of *PIK3CA* gene copy number has been found by array-based comparative genomic hybridization in more than 60% of glioblastomas (Hui et al., 2001); however, this gene is not frequently amplified and/or overexpressed in glioblastomas (Knobbe and Reifenberger, 2001). We have recently reported on amplification and overexpression of the *PIK3C2B* gene at 1q32 in a small subset of glioblastomas (Knobbe and Reifenberger, 2001). In a single gliosarcoma, we found the *AKT1* gene amplified and overexpressed. It is also known that Akt kinases may be activated by various growth factors, including EGF, PDGF, bFGF (basic fibroblast growth factor), and insulin (Burgering and Coffer, 1995; Franke et al., 1995). Thus, it is possible that amplification and overexpression of the *EGFR* and *PDGFRA* (platelet-derived growth factor receptor-$_{\alpha}$) genes in glioblastomas also contribute to aberrant Akt activity in these tumors.

Prognostic Significance of PTEN Alterations in Gliomas

Several recent studies have addressed the issue of *PTEN* gene aberrations as a potential prognostic marker for malignant glioma patients. Sasaki et al. (2001) investigated 72 patients with anaplastic oligodendrogliomas. Allelic losses on 10q involving the *PTEN* locus were detected in 14 tumors, whereas 7 tumors carried a *PTEN* mutation (6 cases) or had lost both *PTEN* alleles (1 case). Allelic loss on 10q was negatively associated with 1p loss and indicative of a poor response to chemotherapy and a significantly shorter survival time (median survival time 15.3 months with 10q loss versus 124.4 months without 10q loss). Similarly, *PTEN* aberrations were associated with significantly shorter survival time of anaplastic oligodendroglioma patients (median survival times of 14.8 months versus 123.4 months) (Sasaki et al., 2001).

In anaplastic astrocytomas (WHO grade III), *PTEN* mutations were also found to be a powerful independent marker for reduced survival time. In a study of 62 anaplastic astrocytomas, *PTEN* mutations were detected in 11 tumors; the median survival times for cases with and without *PTEN* mutation were 4.4 months versus 34.4 months (Smith et al., 2001). Thus, a *PTEN* gene alteration detected in a WHO grade III anaplastic glioma (astrocytoma or oligodendroglioma) is strongly indicative of poor prognosis, that being a life expectancy similar to glioblastoma multiforme.

For glioblastomas, the available data are more complicated. Studies focusing on the demonstration of *PTEN* mutations detected no correlation with the outcome of the disease in glioblastoma patients (Smith et al., 2001; Zhou et al., 1999b). For example, Smith et al. (2001) investigated glioblastomas from 110 patients for *PTEN* mutations. The median survival time of 37 patients whose tumors carried a *PTEN* alteration was 11.7 months, whereas the median survival time of 73 patients without a detected *PTEN* alteration was 11.2 months. On the other hand, 2 independent studies suggested that loss of heterozygosity at microsatellite markers flanking the *PTEN* gene locus on 10q23.3 is a significant predictor of shorter survival among glioblastoma patients (Lin et al., 1998; Tada et al., 2001). In addition, Sano et al. (1999) reported that the *PTEN* mRNA level is an independent prognostic factor for glioblastoma patients. Patients whose tumors had low *PTEN* transcript levels had significantly shorter survival times than did patients with high *PTEN* mRNA expression. These authors additionally found that about two thirds of glioblastomas did not express Pten protein at levels detectable by immunohistochemistry, whereas about one third of the cases were immunohistochemically Pten positive, but with heterogeneous expression patterns. Thus, to precisely evaluate the prognostic significance of *PTEN* aberrations in glioblastomas, one must apparently perform comprehensive analyses at the genetic, epigenetic, transcript, and/or protein levels on a large number of clinically and histologically well-documented cases.

With respect to pediatric gliomas, a study of 39 diffuse astrocytic gliomas (7 grade II, 17 grade III, 15 grade IV) from children suggested that *PTEN* mutation (detected in 3/15 grade IV tumors and 1/17 grade III tumors) was associated with a worse prognosis in the group of children with grade III or IV astrocytomas (median survival times 1 month versus 7 months) (Raffel et al., 1999).

Targeted Molecular Therapy of PTEN-Mutant Cells

As described above, restoration of Pten function in *PTEN*-mutant tumor cells through vector-mediated *PTEN* wild-type gene transfer may result in cell-cycle arrest and/or apoptosis/anoikis of glioma cells in vitro as well as reduced tumorigenicity in vivo (Cheney et al., 1998; Li and Sun, 1998). Furthermore, *PTEN* gene transfer may sensitize glioma cells for treatment with radiation and for CD95L-induced apoptosis (Wick et al., 1999). Wild-type Pten can also sensitize glioma cells to chemotherapy, a capacity that has been linked to the function of Pten in protecting p53 from Mdm2-mediated degradation (Mayo et al., 2002).

Besides the gene transfer approaches, *PTEN*-mutant tumor cells may be targeted by specific pharmacological substances acting as inhibitors of different components of the Pten/PI3K/Akt pathway (Fig. 5). For example, wortmannin and LY294002 are inhibitors of PI3K (Powis et al., 1994; Vlahos et al., 1994), whereas staurosporine has been reported to inhibit the catalytic activity of PDK1 (Hill et al., 2001). Wortmannin and LY294002 are extensively applied as tools to study biological functions of PI3K-dependent signaling in cultured cells. At higher concentrations, however, both substances may inhibit several other kinases as well (for review see Stein, 2001). Staurosporine is a broad-spectrum inhibitor of protein

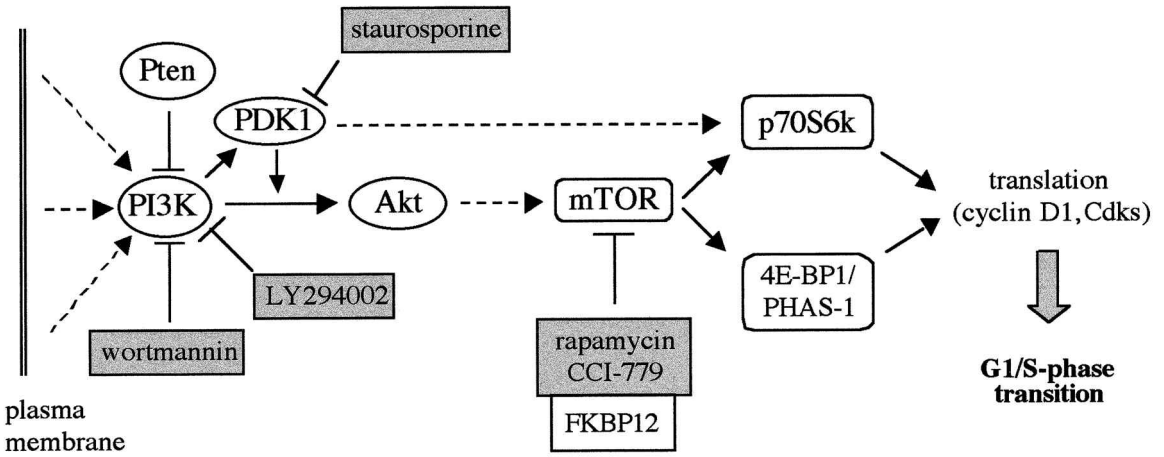

Fig. 5. Graphic representation of the rapamycin/CCI-779–sensitive signaling pathway (modified after Hidalgo and Rowinsky, 2000). Both drugs can bind to FKBP12 (FK506 binding protein 12) and thereby inhibit the kinase activity of mTOR. mTOR inhibition blocks the activity of 2 downstream targets involved in mRNA translation, p70S6k and 4EBP1 (eukaryotic initiation factor 4E binding protein 1/PHAS-1). This decreases the translation of certain proteins important for G_1/S phase progression and results in cell-cycle arrest in G_1. The figure also shows other pharmacologic inhibitors of upstream members of the pathway, such as wortmannin and LY294002 (which irreversibly block PI3K) and staurosporine (which inhibits PDK1).

kinases, including most notably protein kinase C, which is inhibited at an even lower concentration than is necessary for PDK1 inhibition (Hill et al., 2001). Thus, the biological effects induced by wortmannin, LY294002, and staurosporine are concentration dependent and may be due to interference with different signaling pathways. Concerning glioma treatment, administration of either wortmannin or staurosporine has been reported to increase the radiosensitivity of glioma cells in vitro (Kubota et al., 2000; Zhang et al., 1993). In addition, wortmannin promoted gemcitabine antitumor activity in a pancreatic cancer xenograft model (Ng et al., 2001). However, several pharmacological characteristics, such as the instability of wortmannin, the insolubility of LY294002, and the high binding of staurosporine analogs to plasma proteins, may limit the potential of these inhibitors for clinical applications (Gescher, 1998; Stein, 2001).

A promising pharmacologic substance for the treatment of Pten-deficient tumors is the macrolide fungicide rapamycin, an antimicrobial and immunosuppressive agent with potent antitumor activity. CCI-779 is an ester homolog of rapamycin with improved pharmacological properties. Both rapamycin and CCI-779 selectively bind to the FKBP12 (FK506 binding protein 12) and inhibit the protein kinase activity of mTOR (also known as RAFT or FRAP) (Fig. 5) (Hidalgo and Rowinsky, 2000; Schmelzle and Hall, 2000). The inhibition of mTOR interferes with the activity of the 40S ribosomal protein S6 kinase (p70S6k) and the function of EIF4EBP (eukaryotic initiation factor 4E binding protein 1, also known as PHAS-1), which are important factors in ribosome biogenesis and cap-dependent translation, respectively. As a consequence, the translation of mRNAs for a number of key proteins required for cell-cycle progression from G_1 to S phase (for instance, cyclin D1) is inhibited. In addition, rapamycin/CCI-779 prevents cyclin-dependent kinase activation, inhibits pRb (retinoblastoma protein) phosphorylation and accelerates the turnover of cyclin D1, all of which can inhibit G_1/S phase transition (Hidalgo and Rowinsky, 2000; Schmelzle and Hall, 2000). Mutation of *PTEN* is associated with phosphorylation, activation and/or membrane translocation of Akt, mTOR, p70S6k, and 4EBP1 (Mills et al., 2001). In transformed and mitogen-stimulated mammalian cells, the activity of p70S6k and 4EBP1 may be regulated by the Pten/PI3K/Akt pathway through direct phosphorylation of mTOR by Akt (Sekulic et al., 2000). Other authors found evidence that mTOR is a direct target for Akt phosphorylation in mammalian cells, too (Nave et al., 1999). However, a recent study of *Drosophila* indicates that activation of dS6K, the *Drosophila* homolog of mammalian p70S6K, is independent from dPI3K and dPKB (*Drosophila* homolog of mammalian protein kinase B/Akt), but requires dPDK1 as well as dTOR (Radimerski et al., 2002). Similarly, mammalian p70S6K may be phosphorylated and activated directly by PDK1 (Alessi et al., 1998).

Interestingly, 2 recently published studies reported that CCI-779 selectively inhibits the growth of *PTEN*-mutant tumor cells, both in vitro and in the $PTEN^{+/-}$ mouse model (Neshat et al., 2001; Podsypanina et al., 2001). CCI-779 induced apoptosis in *PTEN*-deficient tumor cells that lacked intact p53 or $p21^{waf1}$, whereas cells with intact or reconstituted p53 underwent G_1 arrest but not apoptosis. Thus, depending on the genetic background of the tumor cells, inhibition of mTOR by CCI-779 or rapamycin may have either a cytotoxic or a cytostatic effect. With respect to glioblastoma, CCI-779 likely shows cytotoxic activity because most glioblastomas have altered the Pten/PI3K/Akt pathway and are deficient in p53-mediated growth control. Therefore, CCI-779 appears as a potential new drug for glioblastoma treatment. In a recent experimental study, CCI-779 demonstrated antitumor activity against medulloblas-

toma (DAOY) and glioblastoma (U-251 MG) xenografts in nude mice (Geoerger et al., 2001). As a highly lipophilic substance, CCI-779 is able to penetrate the blood-brain barrier. Early clinical trials on patients with different types of malignant cancers indicate that the drug shows antitumor activity at relatively nontoxic doses (Hidalgo and Rowinsky, 2000). However, CCI-779 is metabolized by the cytochrome P450 system that is inducible by several of the anti-epileptic drugs commonly taken by glioma patients. It may therefore be necessary to administer relatively high doses of CCI-779 for the treatment of malignant gliomas, which in turn increases the risk of toxic side effects. Thus, the clinical significance of CCI-779 for glioma therapy remains to be demonstrated.

Conclusions

The knowledge about *PTEN* aberrations in various types of cancers and the understanding of the function of Pten in normal and neoplastic cells have advanced at an impressive pace over the past 4 years. With respect to gliomas, inactivation of Pten signaling, either by mutation, deletion, or loss of expression of *PTEN* itself or by aberrations of other genes involved in the Pten/PI3K/Akt signaling pathway, appears to be important for the malignant progression to glioblastoma. Aberrant Pten/PI3K/Akt signaling may contribute to several characteristic biologic features of glioblastoma, such as loss of cell-cycle control and uncontrolled proliferation, escape from apoptosis, brain invasion, and aberrant neoangiogenesis. From a diagnostic point of view, the molecular detection of *PTEN* gene alterations or aberrations in other Pten/PI3K/Akt pathway genes is an ominous prognostic sign. However, it may turn out that glioma cells defective in Pten/PI3K/Akt signaling show selective susceptibility to molecular therapeutic regimens that specifically inhibit key effector proteins of the Pten/PI3K/Akt pathway.

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APPENDIX A

Selected World Wide Web Addresses Related to PTEN

OMIM 601728:
http://www.ncbi.nlm.nih.gov:80/entrez/dispomim.cgi?id=601728

PTEN Genomic Sequence (GenBank accession no. AF067844):
http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=nucleotide&list_uids=4240386&dopt=GenBank (further genomic sequence under GenBank accession No. NT_030085)

PTEN Promoter Sequence (GenBank accession no. AF406618):
http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=nucleotide&list_uids=15529617&dopt=GenBank

PTEN mRNA Sequence (GenBank accession no. U93051):
http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=nucleotide&list_uids=1916351&dopt=GenBank (further mRNA sequences under GenBank accession nos.U92436 and NM_000314)

psiPTEN Pseudogene Sequence (GenBank accession no. AF040103):
http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=nucleotide&list_uids=2766242&dopt=GenBank (further *psiPTEN* pseudogene sequence under GenBank accession no. AL356489)

PTEN NCBI Locus Link 5728:
http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=5728

PTEN UniGene Cluster Hs.10712:
http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Hs&CID=10712

PTEN Gene Card:
http://bioinfo.weizmann.ac.il/cards-bin/carddisp?PTEN&search=PTEN&suff=txt

PTEN Germline Mutations (Human Gene Mutation Database):
http://archive.uwcm.ac.uk/uwcm/mg/search/6022948.html