REVIEW PAPER/*ARTYKUŁ POGLĄDOWY*

The role of the *PTEN* gene in malignant gliomas

Znaczenie genu PTEN w glejakach złośliwych

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Abstract

This article focuses on the latest data about the role of the gene for phosphatase and tensin homologue located on chromosome 10 (*PTEN*) in malignant gliomas. *PTEN* acts as a tumour suppressor gene and plays a critical role in cell cycle progression, angiogenesis, migration, invasions and stem cell regulation. Furthermore, there is an interaction with other tumour suppressor genes. We discuss the role of miRNAs in modulating *PTEN* expression and also *PTEN*'s role in the nucleus.

Key words: *PTEN*, *AKT*, glioma.

Streszczenie

W artykule przedstawiono najnowsze dane dotyczące roli genu dla homologu fosfatazy i tensyny (*PTEN*) w glejakach złośliwych. *PTEN* działa jako gen supresorowy nowotworzenia i odgrywa kluczową rolę w przebiegu cyklu komórkowego, w angiogenezie, migracji, inwazyjności i regulacji komórek macierzystych. Wchodzi ponadto w interakcje z innym genami supresorowymi nowotworzenia. W artykule omówiono znaczenie miRNA w modulowaniu ekspresji *PTEN* oraz rolę, którą *PTEN* odgrywa w jądrze komórkowym.

Słowa kluczowe: *PTEN*, *AKT*, glejak.

Introduction

Malignant gliomas are the most common type of primary brain tumours. Treatment remains difficult despite the large amount of research undertaken. The median survival from the time of diagnosis is one year on average for glioblastoma multiforme (GBM) and three years for anaplastic astrocytomas [1,2]. The most aggressive GBM is characterized by uncontrolled proliferation, high levels of neovascularization, diffuse infiltration, resistance to death-inducing stimuli and necrosis [3]. Several genetic alterations have been found in malignant gliomas such as gene mutations, amplifications, loss of heterozygosity or deletions that give promise to the development of more targeted and effective therapies.

Phosphatase and tensin homologue located on chromosome 10 (10q23.3) (*PTEN*) was identified in 1997 as a tumour suppressor gene [4,5]. *PTEN* has been found mutated in a large number of cancers at high frequency, including brain tumours [6,7]. *PTEN* mutations have been found in as many as 20-40% of GBM, mainly in primary GBM [3,8]. The PTEN structure reveals an N-terminal phosphatase domain, a C2 domain and the C-terminal tail and is a dual protein and lipid phosphatase. The identification of PTEN mutants, which have defects in either lipid or both lipid and protein phosphatase activities, has made it possible to further

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clarify PTEN's function. The protein contains a tensin-like domain as well as a catalytic domain, dephosphorylating serine, threonine, and tyrosine phosphorylated proteins [9]. As a lipid phosphatase, PTEN dephosphorylates the intracellular levels of phosphatidylinositol (3,4,5)-triphosphate (PIP_3), a product of phosphoinositide-3-kinase (PI3K), to create phosphatidylinositol (4,5)-bisphosphate (PIP_2). Moreover, PTEN is the sole central negative regulator of PI3K signalling because no other protein compensates if there is a loss in its function. PTEN disruption leads to PIP_3 accumulation which activates a cascade of signalling molecules including the phosphatidylinositol-dependent kinases (PDKs), the serine/threonine kinases AKT/protein kinase B, S6 kinase, and mTOR, as well as small GTPases Rac1 and Cdc42. One of the most studied PTEN downstream effects is the activation of AKT, which regulates angiogenesis via activation of hypoxia-inducible factor-1 (HIF-1) and VEGF, cell migration and invasion through matrix metalloproteinases (MMP) regulation, cell cycle progression through down-regulation of the G1 cell cycle inhibitor, p21 and p27, cell survival through the inhibition of proapoptotic factors such as BAD, BIM, FAS ligand and caspase 9, cell growth via the activation of mTOR and corticogenesis via the mediation of insulin-like growth factor-1 (IGF-1) [10,11] (Fig. 1). Furthermore, loss in PTEN function results in high levels of insulin-like growth factor binding protein-2 (IGFBP-2) expression via the loss of its lipid phosphatase activity [9]. IGFBP-2 can act as a growth stimulator and there are reports of a positive correlation between IGFBP-2 expression and grade of gliomas [12,13].

PTEN and p53

P53, a crucial tumour suppressor gene, has an important role in the transcriptional activation of genes involved in cell cycle control, DNA repair, senescence, angiogenesis, and apoptosis. P53 mutations are the most frequent in human cancers [14]. Several studies have reported that *PTEN* and p53 are linked up. It has been shown that *PTEN* transcription can be regulated by p53 because the *PTEN* promoter contains a p53-binding site element that is required for the transactivation of *PTEN* [15]. Loss of p53 resulted in reduction of *PTEN* expression and increased ultraviolet-induced AKT activation in 293T glioma cells as demonstrated by Chappell *et al*. [16]. On the other hand, p53 may

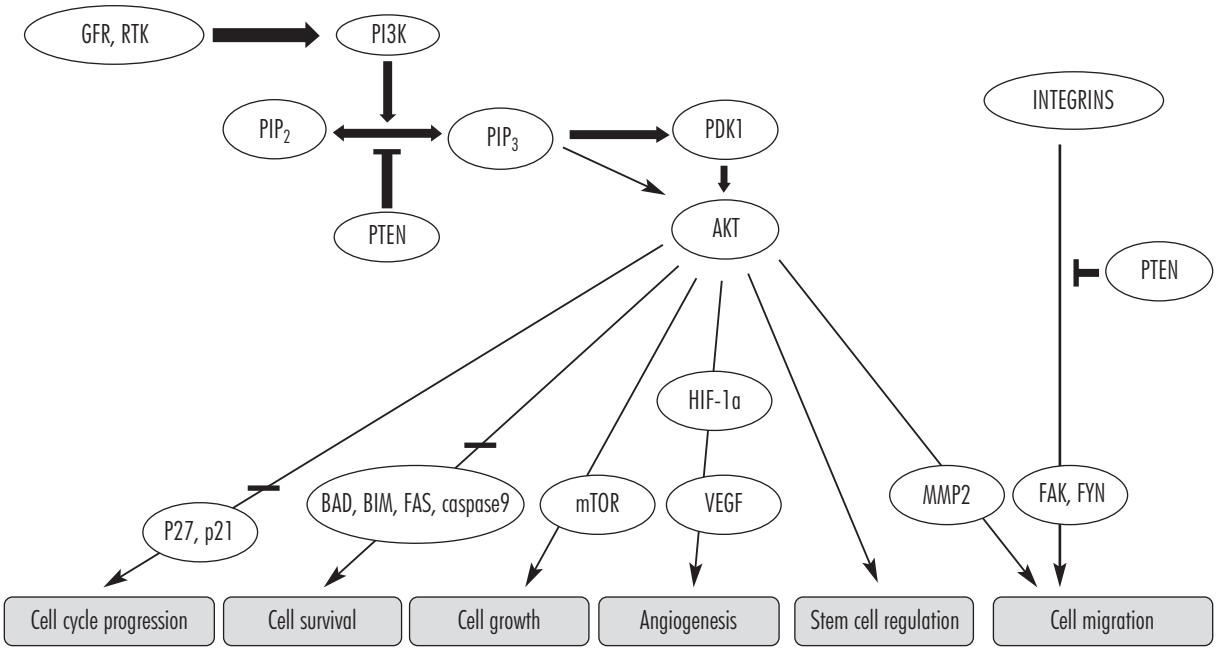

Fig. 1. Representation of the Pten/PI3K/Akt signalling pathway. PI3K can be activated by growth factor receptors and tyrosine kinase receptors. PI3K induces the production of PIP3 which activates AKT. PTEN antagonizes PI3K signals. AKT regulates cell cycle progression (down-regulation of p21 and p27), cell survival (via inhibition of BAD, BIM, FAS ligand and caspase 9), cell growth (via activation of mTOR, a growth regulator), angiogenesis (activation of HIF-1 and VEGF), stem cell and cell migration and invasion through the regulation of MMPs. PTEN dephosphorylates focal adhesion kinases (FAK) and SRC family kinases (FYN)

down-regulate PTEN partially by activating caspases under stress induced by proteasome inhibition [17].

PTEN and miRNAs

MicroRNAs (miRNAs) are small (18–25 nt) non-coding RNAs implicated in the pathogenesis of various malignancies by regulating the expression of several tumour suppressors and oncogenes [18]. MiR-19a and miR-21 have been reported to specifically target and to down-regulate *PTEN* [18,19]. Furthermore, *PTEN* is regulated by miR-214, which directly binds the *PTEN* 3'-UTR leading to inhibition of *PTEN* translation and subsequent activation of the PI3K/AKT pathway [20]. Huse *et al*. reported that miR-26a, another down-regulator of *PTEN* expression, is usually amplified in gliomas. *PTEN* suppression by miR-26a in a murine glioma model enhanced de novo tumour formation and precluded loss of heterozygosity and the *PTEN* locus [21].

Nuclear PTEN

Research into PTEN revealed that apart from its cytoplasmic localization, it can be found mainly in the nucleus of normal cells [22,23]. Subsequent research revealed that loss of the nuclear PTEN can be found in a variety of tumours [24,25]. This leads to the belief that nuclear PTEN may be involved in neoplastic transformation and that might modulate its activity. Nevertheless, PTEN does not contain any obvious nuclear import/export signal. Several mechanisms have been implicated for the nuclear import of PTEN, such as simple diffusion [26], phosphorylation-dependent transfer [27], active transport through NLS-like signals [28], interaction with the major vault protein mediated by Ca^{2+} signalling [29] and monoubiquitylation of PTEN at K289 [30].

One of the functions of nuclear PTEN is the control of AKT activity, since PI3K, PDK1 and activated AKT can be found in the nucleus. Furthermore, nuclear PTEN also induces a G0-G1 arrest by decreasing the cyclin D1 levels [31]. Liu *et al*. recently found that nuclear PTEN can suppress anchorage-independent growth and facilitate G1 arrest in U251MG glioma cells without inhibiting AKT activity [32]. Moreover, Gil *et al.* showed that apoptotic stimulation, via TNFa in U87MG glioma cells, increased the nuclear accumulation of PTEN. The overexpression of catalytically active nuclear PTEN enhanced cell apoptotic responses in U87MG glioma cells [33].

Specific functions of *PTEN*

Cell cycle progression

Loss of *PTEN* function in astrocytes results in increased proliferation [49]. Several studies have reported that re-expression of *PTEN* in *PTEN*-deficient glioblastoma cell lines suppresses proliferation *in vitro* [33-36]. Cell cycle arrest in the G1 phase is the mechanism for the proliferation defect induced by *PTEN* in most glioblastoma cells [35,37]. Activation of the PI3K pathway has been shown to regulate cell-cycle progression directly through AKT-mediated phosphorylation of cell cycle inhibitors, such as p27, p21 and GsK3β [39,40]. Id-1 is a novel *PTEN* inhibitor, acting by down-regulation of p53 expression, that activates the PI3K/AKT signalling pathway and affects its downstream effectors, the Wnt/T-cell factor (TCF) pathway and p27Kip1 phosphorylation and its cytosolic retention [41].

Angiogenesis

Mutation of *PTEN* has been observed mainly in high grade gliomas in which neovascularization is present. Consequently, there has been an effort to elucidate *PTEN*'s role. Hypoxia, VEGF, acidic fibroblast growth factor, IL-6 and IL-8 are known inducers of angiogenesis, whereas angiostatin, endostatin, thrombospondin 1 (TSP1) and endothelial monocyte-activating polypeptide 2 are suppressors [42]. Overexpression of AKT1 in endothelial cells of adult mice resulted in formation of pathological blood vessels. Wen *et al*. reported that the reconstitution of wild-type *PTEN* in U87MG glioma cells lines dramatically decreased tumour growth in vivo and prolonged survival in mice implanted intracranially with these tumour cells, but had no effect on in vitro proliferation. *PTEN* reconstitution diminished phosphorylation of AKT within the *PTEN*-reconstituted tumour, induced TSP1 expression, and suppressed angiogenic activity. Using an inactive mutant of *PTEN* they found that the lipid phosphatase activity of PTEN regulates the angiogenic response *in vivo* [43]. Su *et al*. showed that *PTEN* induced the transactivation of p53 and increased

the expression of p53 target genes [44]. P53 is one transcription factor that up-regulates TSP-1 [45].

The tumour vasculature may also be directly influenced by the fluctuating hypoxic environment. HIF-1, a key regulator of the cellular response to hypoxia, is a heterodimeric transcription factor composed of the nearly ubiquitous HIF-1α and its dimerization partner HIF-1β [46]. Under non-hypoxic conditions, HIF-1α is subject to ubiquitination and proteosomal degradation [47]. However, under hypoxic conditions HIF1α is stabilized and forms a dimer with HIF-1β to become an active transcription factor [48]. PI3K/AKT signalling modulates the HIF-1α pathway. It has been found that AKT activation induces VEGF and HIF-1 expression through its two downstream molecules HDM2 and p70S6K1. VEGF and HIF-1 are the mediators that transmit PI3K-induced oncogenic signals for tumour growth and angiogenesis. Consequently, loss of *PTEN* can increase HIF-1 activity in glioma cell lines [48-50]. Emerling *et al.* found that the phosphatase action of the nuclear PTEN is required for the repression of HIF-1 transcriptional activity through the inactivation of Forkhead transcription factor 3a (FOXO3a). FOXO3 is a negative regulator of HIF-1 transcriptional activity by interfering with the ability of p300 to serve as a transcriptional coactivator [51].

Increased glycolysis is characteristic of malignancy. Beckner *et al.* reported that glycolytic enzymes were abundant and some were increased in pseudopodia formed by U87 glioma cells [52]. Activation of AKT, a critical downstream target of *PTEN* signalling, triggers enhanced glycolytic activity and aerobic glycolysis that produce lactate and pyruvate [53]. Lactate is also a known instigator of cytokines and growth factors such as VEGF, TGF-β, and IL-1. Lactate stabilizes HIF-1α even in the presence of oxygen because lactate and pyruvate bind to and inhibit the HIF prolyl hydroxylases that would otherwise hydroxylate HIF-1α and mark it for rapid degradation [54,55]. Hunt *et al.* reported that accumulated lactate appeared to convey the impression of "metabolic need" for vascularization even in well-oxygenated and pH-neutral conditions [56]. This constitutes another possible role of *PTEN* in angiogenesis through the regulation of cell metabolism.

Migration and invasion

Tumour invasion involves cell migration from the primary tumour site to distant normal tissue. Tumour cells have the propensity to adhere to the extracellular matrix (ECM) and degrade it with proteolytic enzymes [57]. *PTEN* over-expression was shown to be able to inhibit the migration and spreading of U87MG and DBTRG glioblastoma cells. Focal adhesion kinase (FAK) was proposed as a potential substrate for PTEN [58]. Furukawa *et al.* showed that PTEN significantly decreased cell migration in both U251 and U373 glioma cells by decreasing the phosphorylation levels of FAK. Furthermore, PTEN decreases the levels of Cdc42-GTP-binding protein and Rac-GTP, which are directly related to the motile activity of cells. Regarding tumour cells' ability to regulate the ECM, there is an inhibitory effect of PTEN on the proteolytic activity of MMP [59].

Liliental *et al*. reported that PTEN influences migration by regulation of Rac1 and Cdc42 in a lipid phosphatase-dependent manner [60]. Nevertheless, in microinjected glioblastoma cell monolayers, the C2 domain of PTEN alone was able to block cell migration, suggesting that in order to inhibit migration, PTEN requires protein phosphatase activity to autodephosphorylate its C-terminal phosphorylation sites [61]. *PTEN* G129E, lacking PtdIns(3,4,5)P3 phosphatase activity, could inhibit cell motility as efficiently as the wild-type enzyme, when expressed through microinjection of expression constructs into PTEN null glioblastoma cells [61]. A similar conclusion was supported by *in vivo* studies in the early chick embryo [62]. Furthermore, *PTEN* regulates integrin-directed migration not in a PI3K-dependent but in a protein phosphatase-dependent manner, through the control over the activity of SRC family kinases (specifically FYN) [63].

Protein kinase C type i (PKCi), which is a member of the atypical protein kinase C and downstream mediator activated by the PI3K pathway, has an important role in cell motility and invasion by repressing the expression of mRNA for RhoB. RhoB is a member of the Rho GTPase family of proteins that regulates a variety of cellular processes including actin organization, proliferation and differentiation [64]. Expression of RhoB in U87 and A172 glioma cells significantly reduced their motility. The inhibition of PI3k from PTEN results in an increase in RhoB levels [65].

Stem cell regulation

Cancer stem cells have been implicated as initiators for the development of brain tumours. Dirks and colleagues initially showed that human GBM xenografts into immunodeficient mice have this identifiable subset

of cancer-propagating cells or cancer stem cells [66]. In adult people, stem cells represent a relatively inactive subpopulation, which can enter the cell cycle upon growth factor stimulation to replenish specific cellular populations and then exit the cycle (G0 cell cycle state). Recent studies have shown that mutations in the stem/progenitor compartment account for the majority of these tumours and neural stem/progenitor cells as cancer-initiating cells have been identified in astrocytoma mouse models. Furthermore, astrocytoma induction occurs efficiently in embryonic, early postnatal, and adult mice dependent on stem/progenitor cell targeting of the tumour suppressors. Deletion of *PTEN* has been shown to regulate neural stem cell self-renewal [67] and proliferation [68,69]. Sinor *et al*. demonstrated that overexpression of AKT-1, a critical downstream target of PTEN signalling, resulted in enhanced self-renewal of cortical progenitor cells [70]. Furthermore, Groszer *et al*. reported that the loss of *PTEN* enhances G0 cell cycle exit and self-renewal capacity and decreases growth factor dependence [69].

PTEN appears to influence neuronal and astrocyte differentiation [71]. Yue *et al.* reported that PTEN is involved in the differentiation of cerebellar Bergmann glia cells [72]. Chang-Hyuk Kwon *et al*. developed genetic mouse models for de novo GBM that support the idea that in the presence of NF1, p53 and *PTEN* heterozygosity, *de novo* high-grade gliomas appear without requisite transition through low-grade status, undergoing LOH at NF1 and p53, yet retaining *PTEN* heterozygosity. *PTEN* heterozygosity confers haplo-insufficiency for *de novo* high-grade tumour formation [73].

Prognostic significance of *PTEN* mutation

Clinical studies have revealed that *PTEN* mutation in glioblastoma has no correlation with survival [74]. Nevertheless, in anaplastic oligodendrogliomas and astrocytomas there was a positive correlation between *PTEN* alterations and poor prognosis [74,75]. Furthermore, elevated AKT activity has been associated with poor prognosis [76]. Paediatric patients harbouring *PTEN* mutation in tumours have poorer prognosis [77]. Thorarinsdottir *et al*. reported that deficient *PTEN* expression was associated with worse overall survival in childhood high grade gliomas [78].

Conclusions

PTEN has been established as an important tumour suppressor gene. Understanding of the integration of biochemical pathways involved in both tumorigenesis and cancer suppression is a key to the development of improved pharmacological treatment strategies for cancer. The continuously improving knowledge of the PTEN/PI3K/AKT pathway can lead to the development of promising and effective molecular therapeutic regimens that specifically inhibit key effector proteins in this pathway and hold promise to an effective glioma treatment.

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Disclosure

Authors report no conflict of interest.

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