PI3K/Akt and Stat3 signaling regulated by *PTEN* control of the cancer stem cell population, proliferation and senescence in a glioblastoma cell line

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Received May 15, 2012; Accepted July 4, 2012

DOI: 10.3892/ijo.2013.1765

Abstract. Malignant gliomas are the most common primary brain tumor in adults. A number of genes have been implicated in glioblastoma including mutation and deletion of *PTEN*. *PTEN* is a regulator of PI3K-mediated Akt signaling pathways and has been recognized as a therapeutic target in glioblastoma. To achieve potent therapeutic inhibition of the PI3K-Akt pathway in glioblastoma, it is essential to understand the interplay between the regulators of its activation. Here, ectopic expression of *PTEN* in the U-87MG human glioblastoma-astrocytoma cell line is shown to result in the depletion of glioblastoma stem cells (GSCs) and to cause growth retardation and senescence. These effects are likely to be associated with *PTEN*-mediated cooperative perturbation of Akt and Stat3 signals. Using an *in vivo* rat model of glioblastoma, we showed that *PTEN*-overexpressing U-87MG cells failed to induce tumor formation, while untreated U-87MG cells did so. Furthermore, cells expressing the phosphorylated form of Stat3 were completely absent from the brain of rats implanted with *PTEN*-overexpressing U-87MG cells. Based on these results, *PTEN* appears to function as a crucial inhibitor of GSCs and as an inducer of senescence, suggesting that functional enhancement of the *PTEN* pathway will be useful to provide a therapeutic strategy for targeting glioblastoma.

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Key words: PTEN, cancer stem cell, Stat3, Akt, senescence, proliferation

Introduction

Phosphatase and tensin homolog (*PTEN*) is one of the most frequently lost or mutated of the tumor suppressor genes (1-5). It has a structural motif for a dual specificity protein phosphatase, suggesting that *PTEN* functions to negatively regulate protein kinase signaling cascades, a number of which are implicated in tumorigenesis (6). The lipid phosphatase activity of *PTEN* dephosphorylates phosphatidylinositol 3,4,5 trisphosphate (PIP3), a potent activator of the protein kinase Akt (7). Loss of *PTEN* function thus results in the stimulation of cell growth and survival, which are induced by the de-repression of the PI3K/Akt pathway (8,9). However, recent reports suggest that *PTEN* also has PI3K/Akt-independent activities (10). For example, a phosphatase-inactive *PTEN* mutant still retains residual tumor suppressive activity, leading to the hypothesis that *PTEN* has phosphatase-independent tumor suppressor function (11-15).

High-grade malignant gliomas are the most common primary brain tumor in adults. The majority of glioblastomas have histologic characteristics of cells belonging to the astrocytic lineage. Therefore, these tumors are thought to arise from the transformation of astrocytes or their precursors, the neural stem cells (16-19).

The leukemia inhibitory factor receptor β (LIFRβ) and downstream Stat3 signaling pathway supports the self-renewal proliferation of neural stem cells or stimulates the differentiation of neural stem cells into astrocytes (20,21). The enhanced expression and pro-survival activity of Stat3 in a subset of glioblastomas supports the concept that the pleiotropic function of Stat3 signaling contributes to glial cell transformation (22,23). Accordingly, Stat3 associates with the oncoprotein epidermal growth factor receptor III variant in the nucleus to induce glial transformation, whereas it plays a key role in the *PTEN* pathway to suppress malignant transformation of astrocytes (24). These observations indicate that Stat3 plays distinct roles in cell transformation depending on the oncogenic environment.

Recently, there has been a dramatic advance in research on a small subpopulation of cells identified in cancers, which have stem cell properties. The cancer stem cell hypothesis proposes that cancers are derived from a small fraction of cancer stem cells that proliferate through their unique self-renewal ability (25). Cancer stem cells were first identified in

leukemia (26,27). Recently, many investigators have identified them in solid tumors, including those of the breast, brain, pancreas, colon and head and neck (28). The cancer stem cell hypothesis established that cancer therapy must be directed against both the resting cancer stem cells and the proliferating cancer cells (29). Therefore, it is essential to understand the major intracellular signaling pathways governing the characteristics of cancer stem cells. It has become clear that *PTEN* is one of the critical regulators for the development of cancer stem cells and ultimately tumorigenesis.

In this study, we show that ectopic expression of *PTEN* can disturb Akt and Stat3 phosphorylation, which leads to reduced tumorigenicity both *in vitro* and *in vivo*. Furthermore, as demonstrated by the loss of both CD133 expression and neurosphere formation, we found that *PTEN* can suppress the glioblastoma stem cell (GSC) population. Moreover, *PTEN* expression also inhibited cell proliferation and induced senescence in glioblastoma. The data suggest that the occurrence of GSCs, and the proliferation and senescence of glioblastoma, which are closely correlated with *PTEN* expression, are independently regulated by Akt and Stat3 signaling.

Materials and methods

Cell culture and genetic modification. Human glioblastoma-astrocytoma, epithelial-like cell line U-87MG was purchased from ATCC (www.atcc.org). These cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Gibco Invitrogen, Carlsbad, CA, www.invitrogen.com). Neurospheres were formed in DMEM containing Nutrient Mixture F-12 (DMEM/F12, Gibco Invitrogen) supplemented with 20 ng/ml basic fibroblast growth factor (Gibco Invitrogen), 20 ng/ml recombinant human epidermal growth factor (R&D Systems, Minneapolis, MN, http://www.rndsystems.com), and 20 μl/ml N2-supplement (Gibco Invitrogen). A *PTEN*-expressing plasmid was constructed by cloning PCR-amplified *PTEN* cDNA into pLPCX (Clontech, Mountain View, CA, http://www.clontech.com). The *PTEN*-expressing plasmid was transfected into U-87MG cells with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. U-87MG cells that stably express *PTEN*, by chromosomal integration of the PTEN-expressing plasmid, were selected in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 500 μg/ml puromycin (Gibco Invitrogen) for 2 to 3 weeks.

RNA extraction and real-time RT-PCR. Total-RNA was extracted using TRIzol (Invitrogen) and 2-5 μg total-RNA was reverse-transcribed using the SuperScriptII™ First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. Real-time RT-PCR was carried out on cDNAs using the QuantiTect SYBR-Green PCR Kit (Qiagen, Valencia, CA). Reactions were conducted in triplicate using the Exicycler™96 Real-Time Quantitative Thermal Block (Bioneer, Korea, http://www.bioneer.co.kr). For quantification, the expression of target genes was normalized against that of the glyceraldehyde 3-phosphate dehydrogenase gene (*GAPDH*). The PCR primer pairs (sense, antisense) were as follows: *CD133* (cag agt aca acg cca aac ca, aaa tca cga tga ggg tca gc) and *GAPDH* (ggg tgt gaa cca tga gaa, gtc ttc tgg gtg gca gtg at).

Protein extraction and western blot analysis. Cells were washed twice with cold phosphate-buffered saline (PBS), lysed with tissue lysis buffer (20 mM Tris base pH 7.4, 137 mM NaCl, 2 mM EDTA, 1% Triton X-100, 25 mM β-glycerophosphate, 2 mM sodium pyrophosphate, 10% glycerol, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride and 1 mM benzamidine) and clarified by centrifugation at 12,000 x g for 10 min. Whole-cell extracts were prepared and 20-50 μg protein was resolved by SDS-PAGE and blotted using antibodies against PTEN (sc-6817-R, Santa Cruz Biotechnology), Akt (sc-8312, Santa Cruz Biotechnology), p-Akt (#4058, Cell Signaling Technology), p-Stat3 (#9131, Cell Signaling Technology), Stat3 (sc-482, Santa Cruz Biotechnology) and β-actin (sc-47778, Santa Cruz Biotechnology). Immunoreactivity was detected by enhanced chemiluminescence (Amersham Biosciences).

Immunocytochemical staining. Cells were fixed with 4% paraformaldehyde in PBS for 20 min and permeabilized with 0.1% Triton X-100 in PBS for 15 min. After treatment with 1% goat serum for 1 h, cells were incubated with primary antibodies against goat anti-human p-Stat3 or CD133 (1:100 dilution) at 4˚C overnight. Cells were washed with PBS and then incubated with Alexa 488-labeled anti-goat IgG secondary antibody (1:300; Molecular Probes, Eugene, OR) for 2 h.

Senescence analysis. The SA-β-gal staining method was used to analyze senescence. Cells were washed twice with PBS (pH 7.2), fixed with 2% formaldehyde and 0.2% glutaraldehyde in PBS (pH 7.2), and washed in PBS (pH 7.2) supplemented with 1 mM $MgCl_2$. Cells were stained in X-gal solution [1 mg/ml X-gal (Boehringer Ingelheim, Ridgefield, CT, http://us.boehringer-ingelheim.com/)], 0.12 mM $K_3Fe[CN]_6$, 0.12 mM $K_4Fe[CN]_6$, 1 mM $MgCl_2$ in PBS at pH 6.0) at 37˚C overnight and then examined under microscope (CKX41, Olympus, Japan, http://www.olympus-global.com/).

Migration assay. For scratch tests, cells were plated in 6-well plates at 90-100% confluency. A scratch was made at the center of the well with a 200-μl pipette tip. Cell migration was observed under microscope 24 h after wounding. This experiment was repeated three times.

Rat model of glioblastoma. All of the experimental animals were manipulated in accordance with the guidelines of the CHA University Institutional Animal Care and Use Committee (IACUC090012). Adult male Sprague-Dawley rats (n=11) weighing 260-280 g (Orient, Seoul, Korea) were used in this experiment. One million ordinary U-87 MG cells (called WT thereafter) or U-87 MG cells overexpressing *PTEN* (called *PTEN* OE thereafter) in a volume of 2 μl were stereotaxically implanted into one hemisphere of the brain (n=5 WT and n=6 *PTEN* OE) using the following coordinates: AP, +1.0 mm; ML, -3.0 mm and DV, -5.0 mm. Animals were euthanized at 5 weeks following implantation and brains were taken for histological analyses. H&E staining was carried out on 10 μm-thick brain sections, whereas immunohistochemical staining was carried out on 40 μm-thick sections using the following antibodies:

p-Stat3 (1:100, Cell Signaling), PTEN (1:100, Santa Cruz Biotechnology), human-specific nuclei (hNu) (1:200, Chemicon), goat anti-mouse IgG-conjugated Alexa-555 (1:200, Molecular Probes) and goat anti-rabbit IgG-conjugated Alexa-488 (1:200, Molecular Probes).

Statistical analysis. Graphical data are presented as means ± SD. Each experiment was performed at least three times and subjected to statistical analysis. Statistically significant differences between two groups were determined using Student's t-test. A $p<0.05$ was considered significant. Statistical analysis was performed using the SAS statistical package vs. 9.13 (SAS Inc., Cary, NC, http://www.sas.com/).

Results and discussion

PTEN expression results in the depletion of GSCs in glioblastoma. The *PTEN* pathway is known to control normal stem cell maintenance, self-renewal and migration. *PTEN* loss confers increased self-renewal capacity to normal stem cells/progenitors, which can cause the development of cancer stem cells and ultimately tumorigenesis. Expression of the GSC tumor marker CD133 was used to examine the effect of *PTEN* expression on the GSC population. First, the *PTEN*-deficient U-87MG cells (wild-type, WT) were engineered to stably express *PTEN* (*PTEN*-overexpressing, *PTEN* OE) (Fig. 1A). Fig. 1B shows that the overexpression of PTEN significantly reduced the levels of CD133 RNA and protein, suggesting that *PTEN* expression resulted in the depletion of GSCs. As confirmation, the loss of further GSC characteristics was analyzed. Cell migration ability is one of the prominent characteristics of glioblastoma cancer stem-like cells (30), and, as expected, *PTEN* OE cells showed defective migration ability compared to WT (Fig. 1C). In addition, the ability to form neurospheres, another feature of GSCs, was also significantly diminished in *PTEN* OE cells (Fig. 1D). Taken together, these results suggest that *PTEN* expression clearly reduced the population of GSCs among U-87MG cells.

PTEN expression induces growth retardation and senescence of glioblastoma cells. Negative regulation by *PTEN* of PI3K activity and the PI3K/Akt pathway is of critical importance for cell proliferation. To further analyze the tumor suppressive mechanism of *PTEN*, the effect of *PTEN* expression on U-87MG cell growth was examined. As expected, cell counting and MTT assay revealed that *PTEN* expression reduced the proliferation of U-87MG cells (Fig. 2A and B). Cellular senescence, which can be induced by various stimuli, can function as a critical barrier for cancer development and significantly restrict cancer progression. Since proliferation is directly linked to cellular senescence, the effect of *PTEN* expression on the induction of senescence in U-87MG cells was analyzed. Compared with the WT cells, the number of cells positive for β-gal staining was significantly increased in *PTEN* OE at each passage (Fig. 2C). Addition of bpV to the culture medium to inhibit *PTEN* activity reduced the population of β-gal-positive cells in *PTEN* OE, suggesting that cellular senescence is induced by *PTEN* activity (Fig. 2C). Treatment with bpV consistently rescued the growth retardation of *PTEN* OE (Fig. 2D). Taken together, these results indicate that the restoration of *PTEN* activity in glioblastoma cells markedly inhibits cell proliferation and leads to senescence.

PTEN expression in U-87MG cells disrupts Akt and Stat3 signaling. PTEN inhibits the PI3K/Akt signaling pathway by catalyzing the dephosphorylation of PIP3, while the loss of PTEN induces the activation of the PI3K/Akt cascade, which leads to the stimulation of cell growth and survival (7,8). The LIFRβ-Stat3 signaling pathway is also regulated by *PTEN* and functions as a brain tumor suppressor in astrocytes (24). In contrast, Stat3 activation has been described in a subset of glioblastoma, indicating that Stat3 plays distinct roles in cell transformation depending on the oncogenic environment (23,31). To examine whether *PTEN* expression can affect Stat3 and Akt signaling pathways, the phosphorylation level of Stat3 and Akt in *PTEN* OE was compared with that in WT. In response to *PTEN* expression, the level of phospho-Stat3 (p-Stat3) and phospho-Akt (p-Akt) was markedly reduced (Fig. 3A). Dephosphorylation of Stat3 in *PTEN* OE was confirmed by immunocytochemical staining (Fig. 3B). Consistent with the result shown in Fig. 1B, the expression level of CD133 was also significantly decreased in *PTEN*-expressing cells (Fig. 3B). To confirm that the dephosphorylation of Stat3 and Akt was induced by *PTEN*, the phosphorylation status of STAT3 and AKT was examined in *PTEN* OE following treatment with bpV for 24 h. As shown in Fig. 3C, chemical inhibition of *PTEN* activity enhanced Stat3 and Akt phosphorylation, suggesting that Stat3 and Akt signaling is directly regulated by *PTEN*.

Cell growth, senescence and the population of GSCs are independently regulated by Akt and Stat3 signaling pathways in U-87MG cells. Since the expression of *PTEN* in U-87MG cells were shown to modulate Stat3 and Akt signals, we next examined whether Stat3 signals are regulated by Akt pathway in U-87MG. To do this, U-87MG cells were treated with PI3K inhibitor LY294002 and, unexpectedly, Stat3 was only transiently dephosphorylated and rapidly restored to its original level within 60 min after LY294002-mediated inhibition of Akt activity (Fig. 4A). This result suggests that Stat3 signal is not a downstream target of Akt pathway in U-87MG. Since *PTEN* expression resulted in the depletion of the GSC population and caused growth retardation and senescence of U-87MG cells, we speculate that these phenomena might be induced by the disturbance of Akt and Stat3 signaling. To test this hypothesis, Akt activity in U-87MG cells was inhibited by treatment with LY294002 and then the extent of senescence, cell growth and neurosphere formation was examined. As expected, LY294002 treatment specifically induced senescence and severely inhibited cell growth (Fig. 4B and C). In addition, neurosphere formation was completely blocked by the treatment of LY294002 (Fig. 4D). Taken together, these results suggest that the senescence, growth retardation, and depletion of GSCs in U-87MG cells are, at least in part, mediated by the Akt signaling pathway.

Constitutive activation of Stat3 is frequently detected in clinical samples from a wide range of human carcinomas and the established cancer cell lines, suggesting that Stat3 activity is crucial to cell survival and growth (32,33). In addition, the JAK/Stat3 signaling pathway is required for the growth of

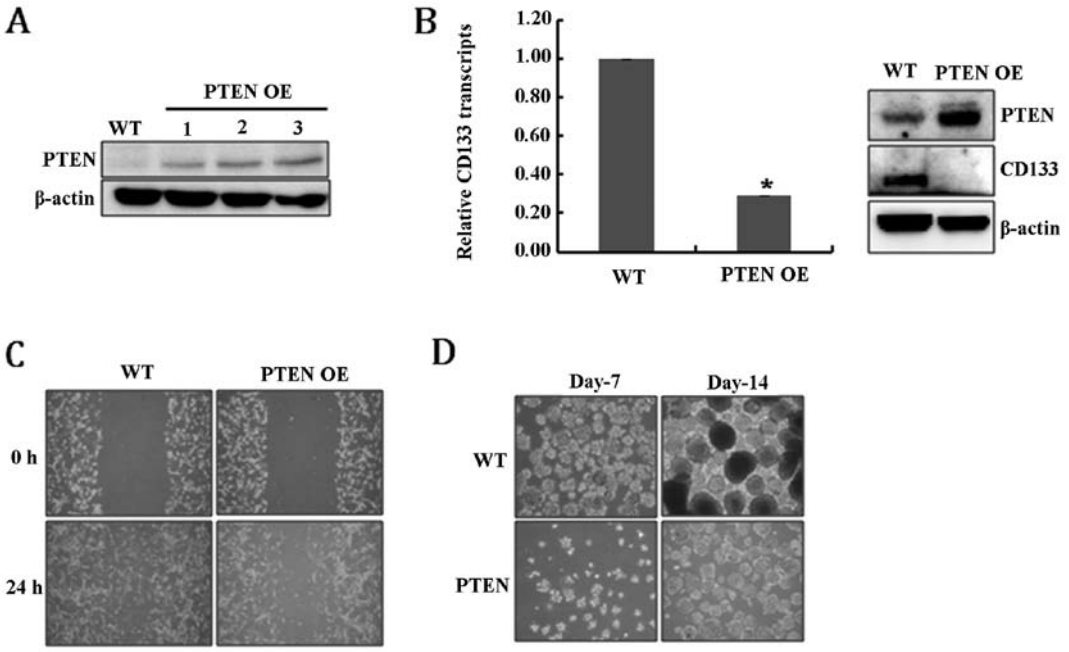

Figure 1. *PTEN* expression results in depletion of GSCs among glioblastoma cells. (A) *PTEN*-expressing U-87MG cell lines were constructed by the stable integration of a *PTEN*-expressing plasmid in U-87MG cells (WT). The expression of *PTEN* in three stable cell lines (*PTEN* OE 1, 2 and 3) was analyzed by immunoblotting. β-actin was used as a loading control. (B) *CD133* mRNA levels in *PTEN* OE and WT were compared by real-time RT-PCR and immunoblot analysis. All values are means ± SD from at least three independent experiments. *p<0.05 based on Student's t-test analysis. (C) Migration of *PTEN* OE was compared with WT by *in vitro* scratch wound healing assay. Photographs were taken 24 h after the wound was made. (D) Representative images demonstrating the reduced neurosphere formation by *PTEN* OE in comparison to WT. Photographs were taken at 7 and 14 days after the transfer of cells into neurosphere-formation medium.

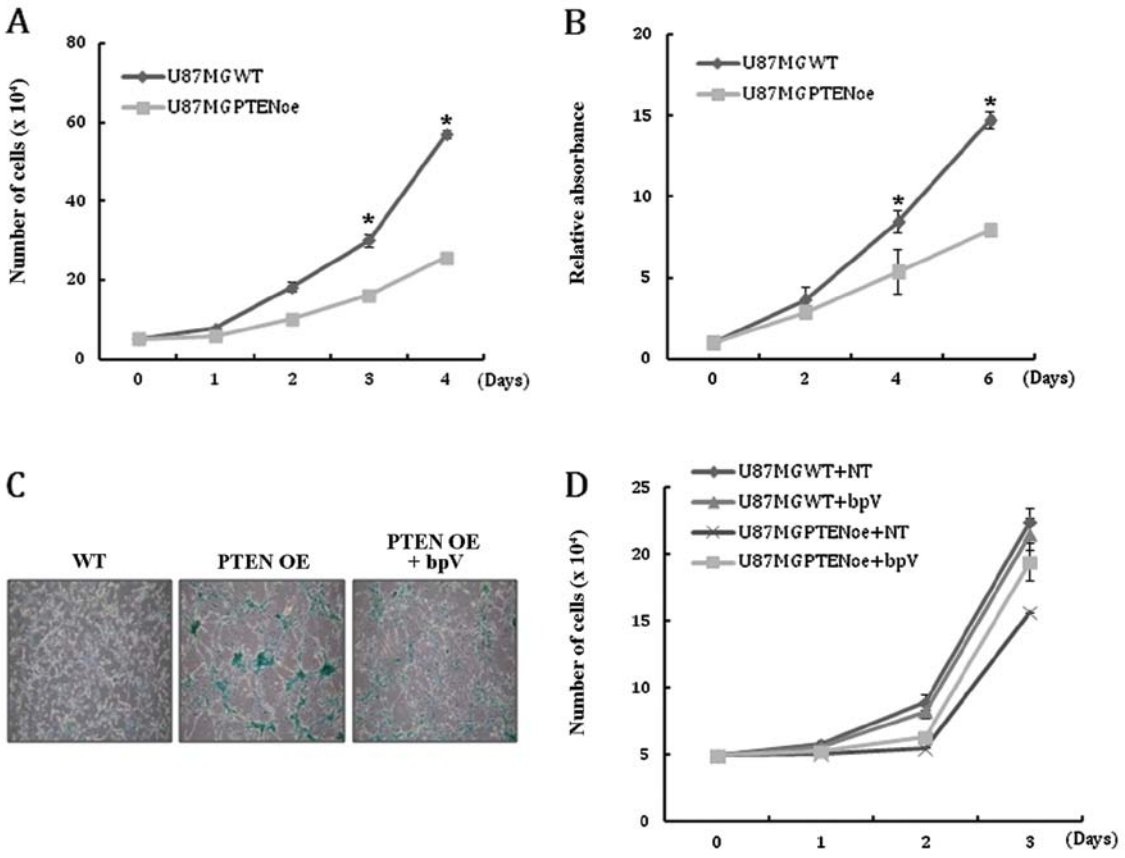

Figure 2. *PTEN* expression induces growth retardation and senescence of glioblastoma cells. Proliferation of WT and *PTEN* OE was analyzed by (A) counting cell numbers every 24 h and by (B) MTT assay every 48 h under monolayer culture conditions. (C) Staining for senescence-associated acidic β-galactosidase (β-gal-positive cells, blue). *PTEN* OE cells were either untreated (*PTEN* OE) or pretreated with 2 μM bpV for 48 h before staining (+ bpV). (D) Proliferation of *PTEN* OE in the presence or absence of 2 μM bpV was examined by counting cell numbers every 48 h. All values are means ± SD from at least three independent experiments. NT, not treated; *PTEN*, *PTEN* OE. All values are means ± SD from at least three experiments. *Indicates significant (p<0.05) results based on Student's t-test analysis.

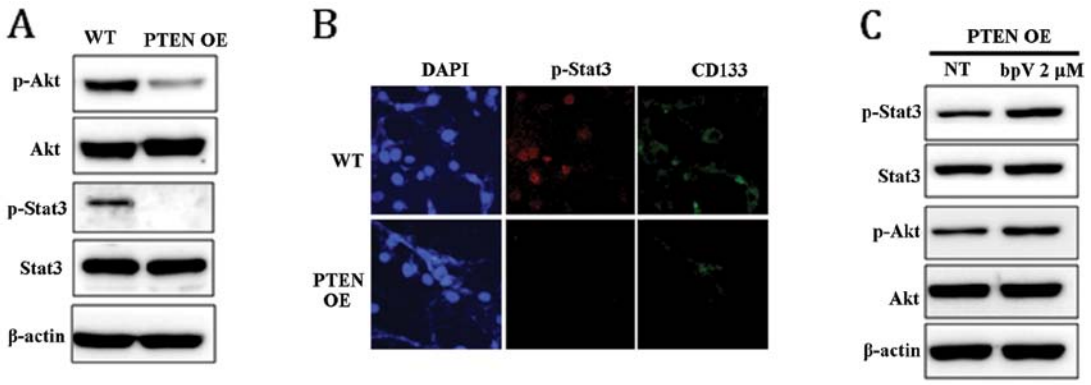

Figure 3. *PTEN* expression in U-87MG cells disrupts Akt and Stat3 signaling. (A) Levels of p-Stat3 and p-Akt in *PTEN* OE were compared with WT by immunoblot analysis. β-actin were used as a loading control. (B) Immunostaining for p-Stat3 and CD133 expression in WT and *PTEN* OE. Cells were counter- stained with DAPI. (C) Immunoblot analysis showing p-Stat3 and p-Akt expression in untreated *PTEN* OE (NT) or cells cultured in the presence of 2 μM bpV for 24 h β-actin were used as a loading control.

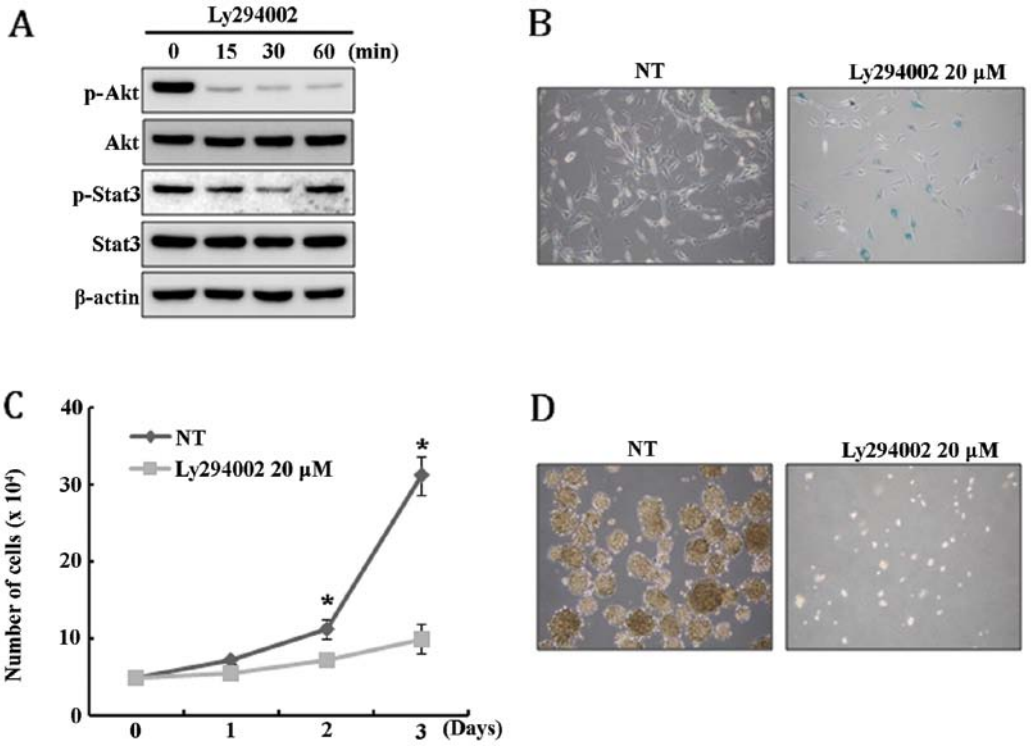

Figure 4. Akt signaling regulates senescence, cell proliferation and neurosphere formation in U-87MG. (A) Immunoblot analysis showing p-Stat3 and p-Akt expression in U-87MG cells harvested at 0, 15, 30 and 60 min after treatment with LY294002 (20 μM). Akt, Stat3 and β-actin were used as a loading control. (B) U-87MG cells cultured in the presence or absence of LY294002 (20 μM) for 2 days were stained for senescence-associated acidic β-galactosidase (β-gal-positive cells, blue). (C) Proliferation of U-87MG cells were examined in the presence or absence (NT) of LY294002 (20 μM) by counting cell numbers 2 and 3 days after seeding. All values are means ± SD from at least three experiments. *p<0.05 based on Student's t-test analysis. (D) Representative images demonstrating the reduced neurosphere formation of U-87MG cells in the presence of 20 μM LY294002, compared to untreated (NT) group. Photographs were taken at 14 days after the transfer of cells into neurosphere-formation medium.

stem-like cancer cells in various tumors (34,35). For these reasons, we decided to examine the effect of inhibition in Stat3 signaling on on cell growth, senescence, and neurosphere formation of U-87MG cells. We found that Stat3 was completely dephosphorylated by treatment with 2 μM WB1066, while phosphorylation of AKT was not affected (Fig. 5A). Interestingly, we observed that cellular senescence, which can be estimated by β-gal staining, was not directly induced by inhibiting the Stat3 signaling pathway, although it caused significant growth retardation (Fig. 5B and C). Treatment with WB1066 completely inhibited the ability of U-87MG to form neurospheres, suggesting that the Stat3 signaling pathway is required for maintaining the cancer stem-like cell population (Fig. 5D). These results demonstrate that Stat3 signaling regulates proliferation and maintenance of the cancer stem-like cell population but is not involved in the senescence of U-87MG cells. Since *PTEN* expression in U-87MG disrupts Akt and Stat3 signals, and that growth retardation, senescence and

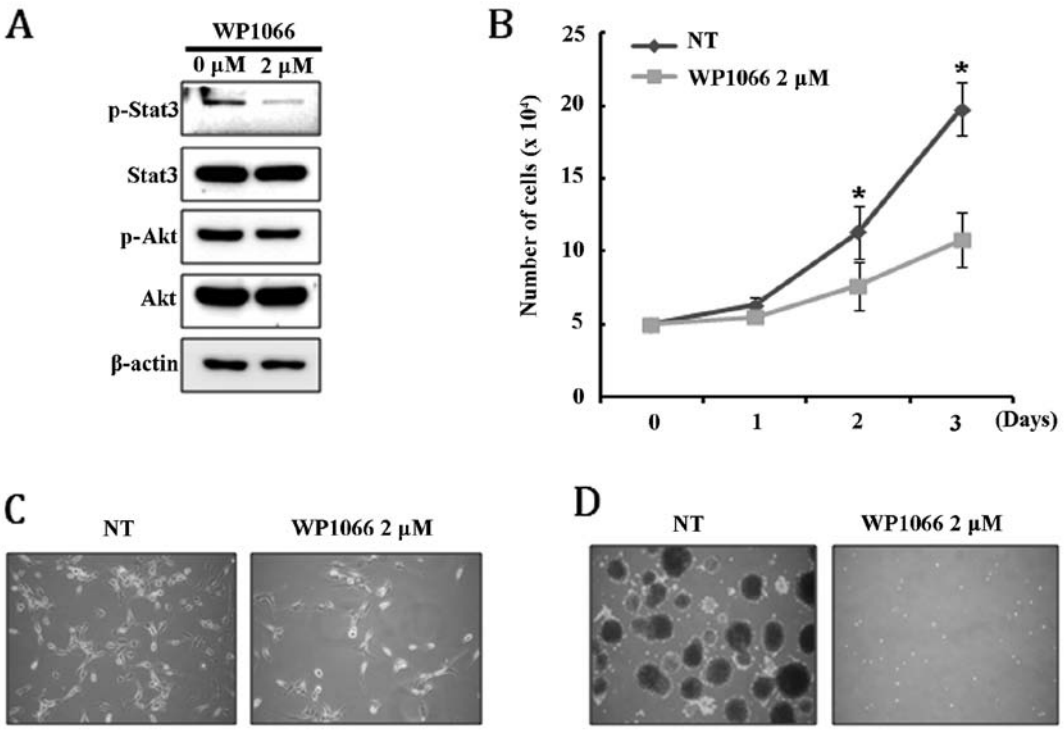

Figure 5. Stat3 signaling regulates cell proliferation and neurosphere formation but not senescence in U-87MG. (A) p-Stat3 and p-Akt expression in U-87MG cells, untreated (0 μM) or cultured in the presence of 2 μM WP1066 for 48 h, were analyzed by immunoblot. Akt, Stat3 and β-actin were used as loading controls. (B) Proliferation of U-87MG cells was examined in the absence (NT) or presence of 2 μM WP1066 by counting cell numbers 2 and 3 days after seeding. All values are means ± SD from at least three experiments. $^{*}p<0.05$ based on Student's t-test analysis. (C) U-87MG cells cultured in the absence (NT) or presence of 2 μM WP1066 for 2 days and then stained for senescence-associated acidic β-galactosidase. (D) Representative images demonstrating the reduced neurosphere formation of U-87MG cells in the presence of 2 μM WP1066 compared to untreated (NT) group. Photographs were taken at 14 days after the transfer of cells into neurosphere-formation medium.

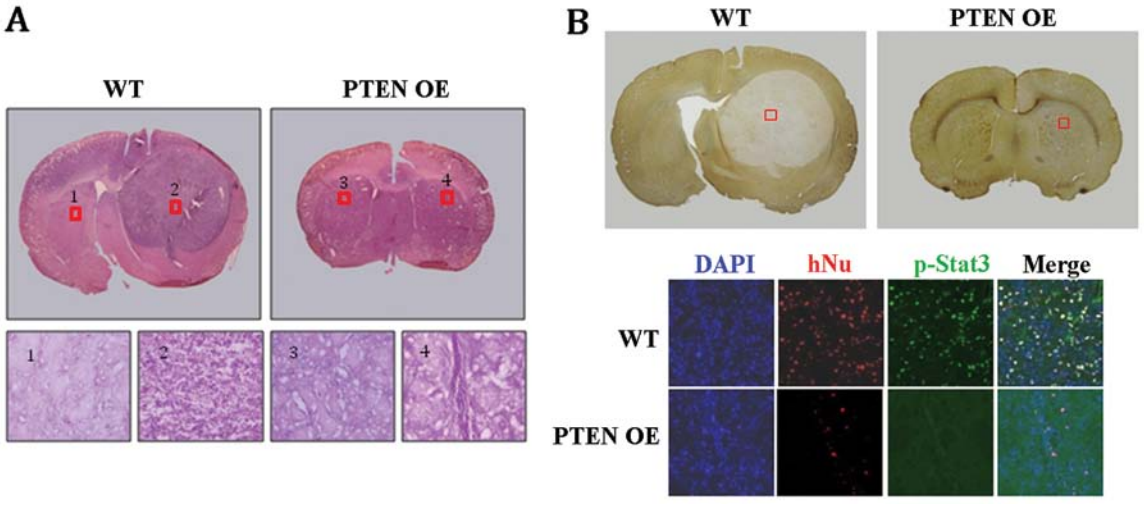

Figure 6. Overexpression of *PTEN* inhibits tumor formation by U-87MG glioblastoma cells *in vivo*. (A) H&E stained sections of brain analyzed at 5 weeks after implantation with WT U-87MG cells or *PTEN* OE U-87MG cells. Numbers 1-4 correspond to higher magnifications (shown in the panels below) of areas contra-lateral (1 and 3) and ipsi-lateral (2 and 4) to the implantation of WT (1 and 2) and *PTEN* OE cells (3 and 4). Note the formation of tumor-like cells in 2, whereas no such cells were formed in 4. (B) Immunohistochemical staining for p-Stat3 at the same stage as in (A). Positive cells were visualized by DAB staining (brown). Note that p-Stat3-positive cells were absent in the area of tumor formation (red box) in WT. Bottom panel shows that hNu- and p-Stat3-positive cells are abundantly detected in WT U87-implanted brain, which are also shown to be co-localized in the Merge image. By contrast, in *PTEN* OE U-87MG-implanted brain, no p-Stat3-positive cells were found and only a very few hNu-postive cells were detected, indicating that minimal numbers of implanted cells were maintained without forming tumors.

depletion of cancer stem cells are caused by *PTEN* expression in U-87MG, we speculate that *PTEN* induces growth retardation and cancer stem cell depletion of U-87MG in conjunction with both Stat3 andAkt signaling pathways, whereas the senescence response of U-87MG cells upon *PTEN* expression is mainly regulated by the Akt signaling pathway.

Overexpression of PTEN inhibits tumor formation by U-87MG glioblastoma cells in vivo. To address whether *PTEN* is involved in the proliferation of GSCs, *PTEN* OE or as controls, WT U-87MG cells were implanted into the brain of 8-week-old rats. Strikingly, no tumor-like structures were detected in any of the *PTEN* OE-implanted group (n=6), whereas robust tumor-like structures were formed in all of the WT-implanted group (n=5) (Fig. 6A and B). Histological analysis of the WT-implanted brains indicated that the tumor-like structures consisted mainly of undifferentiated glioblastoma cells (Fig. 6A; WT 2). In contrast, such cells were not detected in the *PTEN* OE-implanted brains (Fig. 6B; *PTEN* OE 4). Furthermore, immunohistochemical staining revealed that many human-specific nuclear antigen (hNu)-positive cells were detected in the WT-implanted brain sections, indicating that U-87MG glioblastoma cells had proliferated extensively after implantation (Fig. 6B; WT). p-Stat3-positive cells were also common in the area of tumor formation (Fig. 6B; WT). In contrast, in the *PTEN* OE-implanted brains, only a few hNu-positive cells were detected, suggesting that most of the *PTEN* OE U-87MG glioblastoma cells were not maintained after implantation (Fig. 6B; *PTEN* OE in the bottom panel). No p-Stat3-positive cells were detected either in the *PTEN* OE-implanted brains (Fig. 6B; *PTEN* OE in the bottom panel). Taken together, these results strongly suggest that *PTEN* negatively regulates the proliferation of GSCs in U-87MG glioblastoma cells, presumably through the inhibition of the Stat3 pathway.

From our study, we found that analysis of the signaling pathways underlying the tumor suppressor function of *PTEN* in U-87MG cells can provide experimental evidence how the loss of *PTEN* promotes pathogenesis of glial tumors. In particular, identification of the Stat3 and Akt signaling pathways as downstream targets of *PTEN* provides a mechanism by which *PTEN* modulates cell proliferation and senescence and maintenance of the GSC population. Accumulating evidence supports the concept that GSCs, which have stem cell-like properties, are the major cause of the occurrence and maintenance of glioblastoma. In this regard, our results will provide important experimental basis for developing therapeutics to treat glioblastoma in the future.

Acknowledgements

This study was supported by the Korea Science and Engineering Foundation (KOSEF) of the Korean government (MOST) (2010-0003254, 2011-0014084). This study was also supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093821).

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