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The catalytic phosphoinositol 3-kinase isoform p110δ is required for glioma cell migration and invasion

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ABSTRACT

Glioblastoma multiforme (GBM) is a highly invasive and aggressive primary brain tumour in which loss of phosphatase and tensin homologue deleted on chromosome 10 (PTEN), a negative regulator of PI3K signalling, is a common feature. PTEN/PI3K/Akt signalling is involved in the regulation of proliferation, apoptosis and cell migration. Deregulation of PI3K signalling is considered an essential driver in gliomagenesis. However, the role of different PI3K isoforms in glioma is still largely unclear. Here we show that the catalytic PI3K isoform p110δ is consistently expressed at a high level in various glioma cell lines. We used small interfering RNA to selectively deplete p110δ and to determine its tumourigenic roles in PTEN-deficient cells. Interestingly, knockdown of p110δ decreased the cell migration and invasion ability of all GBM cell lines tested. Mechanistically, p110δ knockdown reduced the protein levels of focal adhesion kinase and cell division cycle 42, key regulators of cellular migration. In contrast, pharmacologic inhibition of p110δ by IC87114 or CAL-101 also clearly impaired glioma cell migration but had no obvious effect on the invasion capacity thus pinpointing to possible kinase-dependent and -independent roles of p110δ in glioma pathology. In summary, our data provide novel evidence that in glioma cells p110δ is a key regulator of cell movement and thus may contribute to the highly invasive phenotype of GBM. Isoform specific targeting of PI3Kδ may be beneficial in the treatment of glioblastoma multiforme by specifically inhibiting tumour cell migration capacity.

1. Introduction

Phosphoinositol 3-kinases (PI3Ks) are lipid kinases that mediate signalling transduction through receptor tyrosine kinases (RTK) and G-protein-coupled receptors (GPCR).[1] Upon activation of these receptors at the cellular surface, PI3Ks become activated and convert the plasma membrane lipid phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) to phosphatidylinositol 3,4,5-triphosphate (PtdIns(3,4,5)P_3). It then recruits downstream effector molecules containing pleckstrin homology domains to the membrane, such as protein kinase B/Akt. In contrast, the phosphatase and tensin homologue deleted on chromosome 10 (PTEN), an important tumour suppressor gene, antagonises PI3K activity by dephos-

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phorylating PtdIns(3,4,5)P_3 to PtdIns(4,5)P_2.[2] Concomitantly, loss or mutation of PTEN, which often occurs in tumours like glioblastoma multiforme (GBM)[3] causes accumulation of PtdIns(3,4,5)P_3 mimicking the effect of PI3K activation. PI3Ks have been linked to an extraordinarily diverse group of cellular functions, including cell growth, proliferation, survival and intracellular trafficking.[4,5] Therefore, they are currently attractive therapeutic cancer targets.

The PI3K family is composed of four classes according to their sequence homology and substrate preference.[1,6] All class I_A PI3Ks are heterodimeric molecules composed of an 85 kD regulatory subunit (p85) and a 110 kD catalytic subunit (p110α, p110β and p110δ) whereas p110γ is the only class I_B PI3K isoform which is coupled to one of two non-catalytic subunits, p101 or p87. In contrast to class I_A PI3Ks, PI3Kγ is not activated by RTKs but rather by GPCR through direct interaction with G protein βγ dimers and Ras proteins.[7–9] Class I_A PI3K isoforms, p110α and p110β are ubiquitously expressed, whereas p110δ expression is restricted to leukocytes[10] and to a lesser extent found also in neurons.[11] Interestingly, p110δ is highly expressed in cancer cell lines, including breast,[12] lung[13] and neuroblastoma[14] derived cells. The importance of PI3K signalling in cancer is highlighted by the fact that the PIK3CA gene encoding for p110α isoform is frequently mutated in a constitutively activated or derepressed manner in a number of carcinomas.[15,16] In addition, overexpression of p110β and p110δ affects proliferation and migration in endometrial carcinoma[17] and breast cancer cells,[12] respectively. Hence, PI3Ks contribute significantly to cellular transformation and the development of cancer, however, specific roles of p110 isoforms remain to be characterised in various tumours.

GBM is the most frequent, highly invasive and aggressive primary neoplasm of the human central nervous system. Extensive infiltration of glioma cells into the surrounding normal brain tissue is a characteristic feature of malignant gliomas and a major reason for the very poor prognosis in GBM patients.[18] Clinical and experimental data have shown that glioma cell migration involves several independent mechanisms including glioma resistance to pro-apoptotic stimuli.[19] Thus, aberrantly activated PI3K/Akt/PTEN pathway, which blocks apoptosis, confers survival advantage to these migrating glioma cells. Interestingly, Temozolomide, a pro-autophagic cytotoxic drug, has been demonstrated to have therapeutic benefits in GBM patients.[20] It can overcome apoptosis resistance in glioma cells by arresting them at G_2/M phase of the cell cycle, eventually causing the cells to die from autophagy.[21]

As abnormal PI3K/Akt signalling is commonly found in GBM, it is important to determine the isoform-specific roles of catalytic p110 subunits of PI3K in gliomagenesis, the results of which could potentially lead to the identification of novel therapeutic targets in GBM. Here, we show that p110δ is consistently expressed at a high level in various human GBM cell lines. Employing siRNA mediated inhibition of p110δ expression our results indicate for the first time that the p110δ isoform has an important role in glioma cell migration and invasion presumably through regulation of expression of focal adhesion kinase (FAK) and the Rho GTPase cdc42.

2. Materials and methods

2.1. Materials

Sources of antibodies used are as follows: p110α, p110β, Akt, phospho-Akt (Ser473), FAK, RhoA, Rac1/2/3 and cdc42 (Cell Signaling Technology); p110δ, and β-actin (Santa Cruz Biotechnology); p85α and PTEN (Lab Vision); p85β (GeneTex). Specific inhibitors of p110δ, IC87114 and CAL-101 are from Symansis Limited and Selleck Chemicals, respectively.

2.2. Cell culture

A normal human astrocyte cell line was purchased from ScienCell™ Research Laboratories. Six human glioblastoma cell lines (U-87MG, U-118MG, U-138MG, SW1088, SW1783, and A172) were obtained from ATCC. Further human glioblastoma cell lines (U-343MG, U-373MG, LNZ308, and SK-MG3) and two paediatric glioblastoma cell lines (GBM6840 and GBM2603) were kindly provided by Prof. HK Ng (Prince of Wales Hospital, The Chinese University of Hong Kong). The characteristics of all glioma cell lines are presented in Suppl. Table 1. Cells were maintained in Minimum Essential Medium Alpha (Gibco) supplemented with 10% (v/v) foetal bovine serum (FBS, Gibco).

2.3. Western blotting

All cell lines were starved in serum-free medium for 24 h before protein extraction. Cell pellets were lysed in RIPA buffer and total cell lysates were clarified by centrifugation at 14000 rpm and normalised by Bradford protein assay (Bio-Rad), then separated by 10% SDS–PAGE and transferred to nitrocellulose membranes. Membranes were incubated overnight with primary antibodies and signals were finally visualised by ECL™ chemiluminescent detection (GE Healthcare).

2.4. Small interference RNA

RNA interference (RNAi) synthetic duplexes were purchased from Invitrogen and resuspended to a stock concentration of 20 μM. The target sequence of human p110δ was TCAGC TGCTCCAAAGACATCCAGTA. The corresponding sense and antisense siRNA oligos were 5′-UCAGCUGCUCCAAAGACA UCCAGUA-3′ and 5′-UACUGGAUGUCUUUGGAGCAGCUGA-3′, respectively. RNAi negative control duplex, with similar GC content served as a negative control for the RNAi response. Briefly, 4×10^5 cells were seeded into each well of a 6-well plate. On the next day, cells were transfected with annealed siRNA oligos using Lipofectamine™ 2000 (Invitrogen) according to manufacturer's instructions. Cells were harvested at 24, 48 and 72 h after transfection.

2.5. Real Time RT-PCR

Total RNA was extracted using PureLink™ total RNA purification system (Invitrogen). First-strand cDNA was synthesised from 5 μg of total RNA using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas). Real-time PCR was performed on the ABI PRISM 7500 System by using Platinum® Quantitative PCR SuperMix-UDG (Invitrogen) and TaqMan® Gene Expression

Assays probe and primer mix (Applied Biosystems). Primers used were (gene assay ID): PIK3CD-Hs00192399_m1 and GAPDH-Hs99999905_m1. The thermal cycling conditions were as follows: hold for 10 min at 95 °C, followed by two-step PCR for 40 cycles of 95 °C for 15 s and 60 °C for 1 min. mRNA expression levels were quantified by using the standard curve method. The copy number of each sample was normalised on the basis of its GAPDH content. All samples were performed in duplicate and four independent experiments were performed. Amplification data were analysed with the ABI Prism Sequence Detection Software 2.1.

2.6. Migration assay

Cells were cultured to reach 80–90% confluence. Wound-healing assay was performed using a sterile 200 μL pipette tip to scratch confluent cells to form a wound. Migration of wounded cells was evaluated over a period of 16 h with an inverted Leica DMI4000 B phase-contrast microscope followed by determination of the number of cells migrated into the original wound.

2.7. Invasion assay

The invasiveness of glioma cells was tested *in vitro* using the Boyden chamber invasion assay. Transwell inserts with 8 μm pores were coated with Matrigel (BD Biosciences). Cells were trypsinised and 1×10^5 cells suspended in serum-free medium were added to the insert (top chamber) in duplicate. Medium containing 10% (v/v) FBS was added to each well (bottom chamber) as a chemoattractant. After incubation for 24 h at 37 °C, invaded cells that passed through the membrane onto the lower surface were fixed with buffered formalin and stained with 0.5% (w/v) Toluidine Blue in 2% (w/v) sodium carbonate solution. Invaded cells were enumerated under a light microscope at 200× magnification aided by a 1 mm × 1 mm grid. Cells in ten different fields were counted.

2.8. Statistical analysis

Results are presented as means + S.E.M. Statistical significance was assessed using parametric two-sided Paired *t*-test and Unpaired *t*-test. A *P*-value below 0.05 was considered statistically significant.

3. Results

3.1. p110δ is highly expressed in the majority of human glioblastoma cell lines

We first evaluated the expression levels of class I_A PI3K isoforms in glioblastoma cells. As shown in Fig. 1A, a panel of 12 high grade glioblastoma cell lines was analysed by immunoblotting of cell lysates with isoform-specific antibodies to the PI3K catalytic subunits (p110α, p110β and p110δ) and their corresponding regulatory subunits (p85α and p85β), and protein lysate of normal human astrocyte was used as a reference sample. Overexpression of PI3K p110 catalytic subunits, p85 regulatory subunits and phosphorylated Akt (Ser473) were detected in glioblastoma cell lines compared with normal human astrocyte. Expression of p110α, p110β, p85α and p85β was detected with slight variation amongst all cell lines analysed. However, their p110δ levels were more variable with most glioma cell lines displaying high p110δ protein amounts. Comparable results were observed at the mRNA level (Fig. 1B) where p110δ was much higher than the other two isoforms. In this analysis we used both GAPDH and β-actin for normalisation of mRNA amounts and obtained thereby comparable results (Suppl. Fig. 1). Lastly, PTEN expression was absent in most cell lines except GBM6840, GBM2603 and U-343MG, consistent with high levels of phosphorylated Akt (Ser473) under serum-free conditions. The PTEN protein expressed in U-343MG cells has been shown to be enzymatically inactive[22] because of a point mutation (Suppl. Table 1), thus maintaining high levels of phosphorylated Akt. In subsequent experiments we focused on U-87MG cells, which express all class I_A PI3K isoforms, as a model to investigate the specific function of the p110δ isoform in pathogenesis of glioblastoma, i.e. cell migration and invasion.

3.2. Efficient knockdown of PIK3CD by siRNA in glioma cells

U-87MG cells were transiently transfected with siRNA oligonucleotides targeting PIK3CD (p110δ). Constructs with similar GC content, but unspecific sequences were used as negative controls. Cells were harvested at 24, 48 and 72 h after transfection and the effect of PI3K siRNA on mRNA content and protein expression was evaluated by real-time RT-PCR and Western blot analysis, respectively. PIK3CD mRNA was down-regulated by approximate 70% within 72 h as compared with cells transfected with siControl (Fig. 2). Concomitantly, significant reduction of p110δ protein levels was observed in U-87MG cells 48 and 72 h following transfection with siPIK3CD. The knockdown of PIK3CD was specific as there was no obvious effect on p110α and p110β expressions. To exclude any off-target effects, two other p110δ-specific siRNAs were tested and one siRNA (siPI3KCD-2) gave similar results in p110δ knockdown as well as concomitant down-regulation of cdc42 (Suppl. Fig. 2).

3.3. Down-regulation of p110δ, but not p110α or p110β, effectively inhibits glioma cell migration and invasion potentially through reduction of FAK and cdc42 protein levels

Next, the ability of glioma cells to migrate was assessed by conducting wound-healing experiments (migration assays) followed by quantification of the number of cells migrated into the wound area (Fig. 3A). Interestingly, siPIK3CD transfected cells displayed a substantially decreased migration capacity when compared with siControl cells. Consequently, we tested cell invasion capacity under p110δ knockdown conditions, which is similar to cell migration, but additionally requires proteolysis of the extracellular matrix barrier. As indicated in Fig. 3B, the invasiveness of siPIK3CD transfected cells was significantly reduced when compared with siControl transfected cells.

We further investigated the cytoskeletal-associated network of intracellular signalling proteins controlling cell motility since remodelling of cytoskeletal components is an

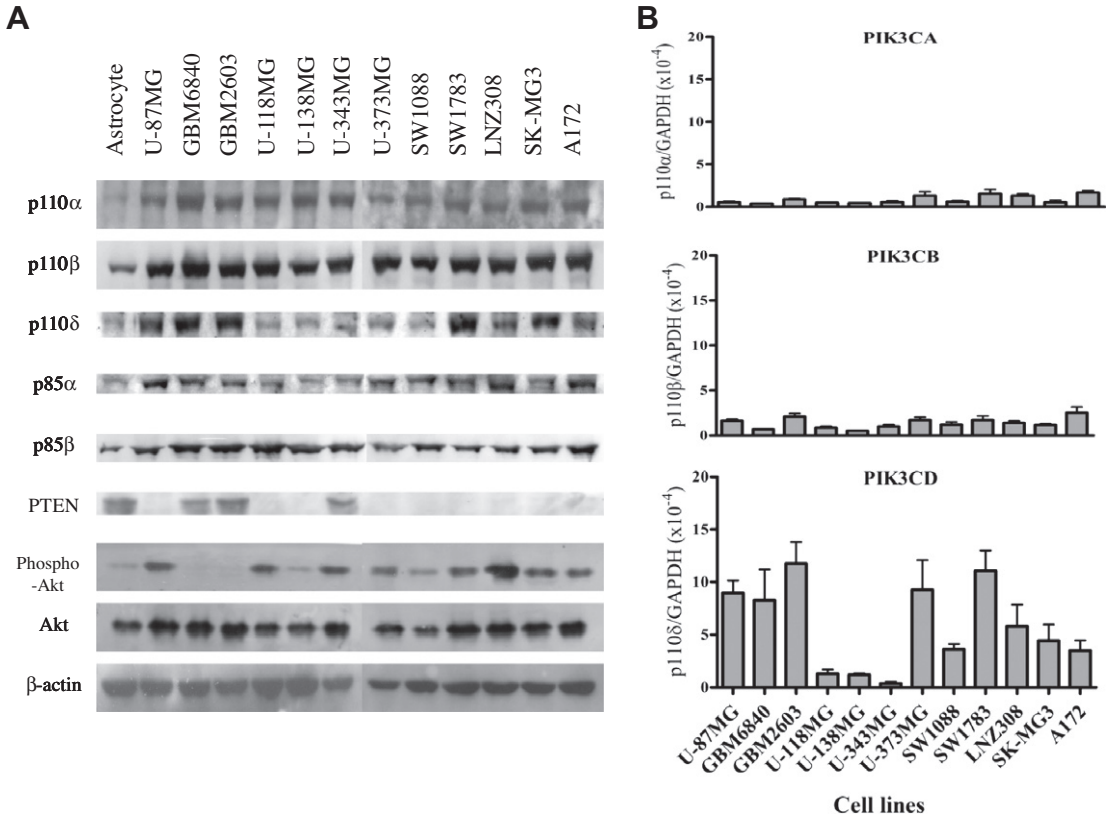

Fig. 1 – Expression of Class I_A PI3K/PTEN in human astrocyte and glioblastoma cell lines. (A) Whole cell lysates were extracted and 50 μg of total protein was analysed for p110α, p110β, p110δ, p85α, p85β, phospho-Akt (Ser473), Akt, and PTEN levels by Western blotting. For p110α and p85α detection, the weak signal was amplified by staining with biotinylated anti-rabbit antibody as secondary antibody, followed by detection with a HRP-conjugated anti-biotin antibody. Gel loading was controlled by immunoblotting for β-actin. (B) Corresponding mRNA expression of Class I_A PI3K isoforms with respect to the GAPDH levels.

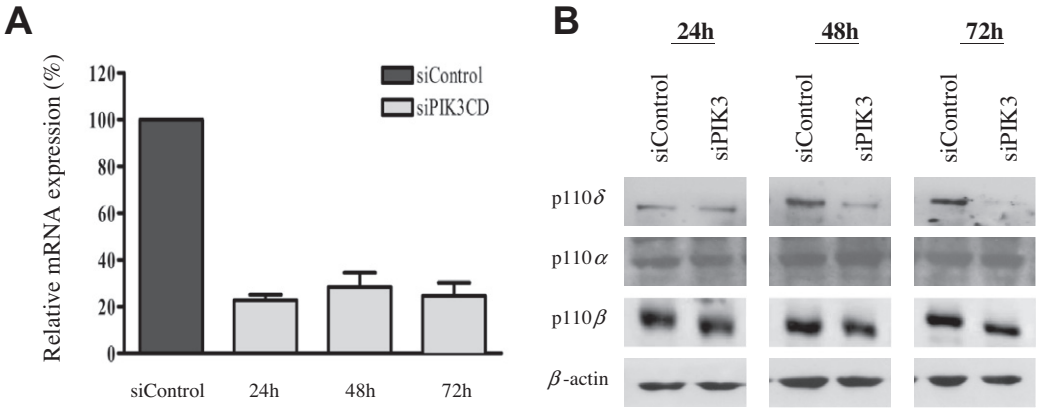

Fig. 2 – Effect of siPIK3CD on p110δ mRNA and protein expression. U-87MG cells were treated with 100 nM siPIK3CD and harvested at 24, 48 and 72 h after transfection. (A) Total cellular RNA was isolated and analysed by quantitative RT-PCR in which GAPDH was used for normalisation. The graph represents the relative levels of down-regulated PIK3CD mRNA compared with that of corresponding siControls. (B) Significant reduction in immunodetectable p110δ upon siRNA treatment was observed at 48 and 72 h, whereas non-targeted PI3K isoform proteins (p110α and p110β) were not affected. The amount of β-actin served as a loading control.

essential step in cancer cell migration and invasion. Remarkably, siPIK3CD treatment resulted in substantial reduction of FAK and cdc42 protein expression (Fig. 3C), key regulators of the above processes impaired following p110δ depletion. In contrast, RhoA and Rac protein levels were not significantly down-regulated following p110δ knockdown. In addition, we did not observe an effect of p110δ knockdown on pAkt (Ser473) levels. The role of p110δ in glioma cell migration

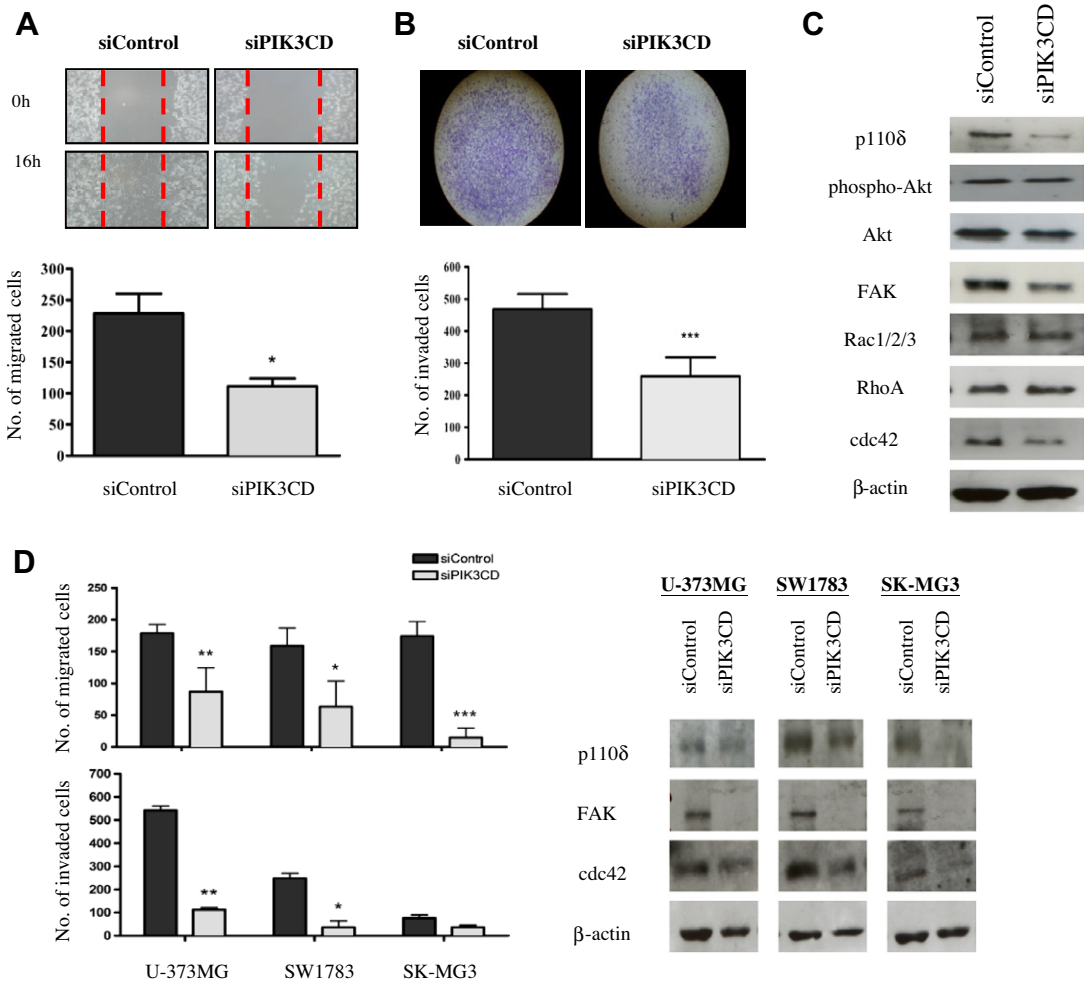

Fig. 3 – The PI3K p110δ isoform is required for efficient glioma cell migration and invasion *in vitro*. U-87MG cells transfected with siPIK3CD showed significant reduction in (A) migration and (B) cell invasion ($^{*}P < 0.05$, $^{***}P < 0.001$). (C) FAK and cdc42 protein expression in these cells was decreased upon p110δ depletion. (D) Comparable effects of siPIK3CD in three additional glioma cell lines which express high p110δ levels ($^{*}P < 0.05$, $^{**}P < 0.01$ $^{***}P < 0.001$).

and invasion was not unique to U-87MG cells, since comparable results were also obtained from the analysis of three other p110δ-expressing glioma cell lines, i.e. U-373MG, SW1783 and SK-MG3 (Fig. 3D). The inhibition of glioma cell migration and invasion by siRNA was not due to a decrease in cell viability since no increased cell death rate or changes in cell cycle distribution were observed (Suppl. Fig. 3). In contrast to the effects of p110δ knockdown, siRNA-mediated depletion of the other class I_A PI3K catalytic subunits, p110α and p110β, failed to significantly affect cell migration and invasion (Fig. 4). Taken together, our data indicate that p110δ is required in an isoform specific/non-redundant manner for efficient cell migration and invasion of glioma cells *in vitro* presumably through regulation of FAK and cdc42 expression and signalling.

3.4. *Pharmacological inhibition of p110δ effectively inhibits glioma cell migration but not invasion*

To confirm the regulation of migration and invasion in glioma cells was particularly due to the PI3K isoform p110δ, we employed a specific p110δ inhibitor, IC87114. Comparable to the phenotype observed upon cellular p110δ depletion, pharmacological targeting of p110δ in glioma cells resulted in their decreased migration capacity when compared with control cells (Fig. 5A). Unexpectedly, the invasion capacity was not affected, albeit slight significant increase in U-87MG cells, after IC87114 treatment (Fig. 5B). Using another p110δ-specific inhibitor, CAL-101, glioma cell invasiveness remained unchanged, but migration capacity was significantly reduced in a comparable manner as observed for IC87114 treatment (Suppl. Fig. 4). Moreover, the molecular effect(s) of pharmacological p110δ inhibition on PI3K/Akt signalling was contrary to siPIK3CD treatment. IC87114 treated glioma cells showed a reduction in phosphorylated Akt (Ser473) protein levels, however, with no significant decrease in FAK or cdc42 levels (Fig. 5C). The slight but significant increase in U-87MG cell invasion may be attributed to the increase in FAK levels in these cells. Taken together, these data indicate that in glioma cells p110δ specifically and positively regulates cell migration and invasion (FAK and cdc42 decreased) since cellular depletion of p110δ negatively impacts on both processes critical for

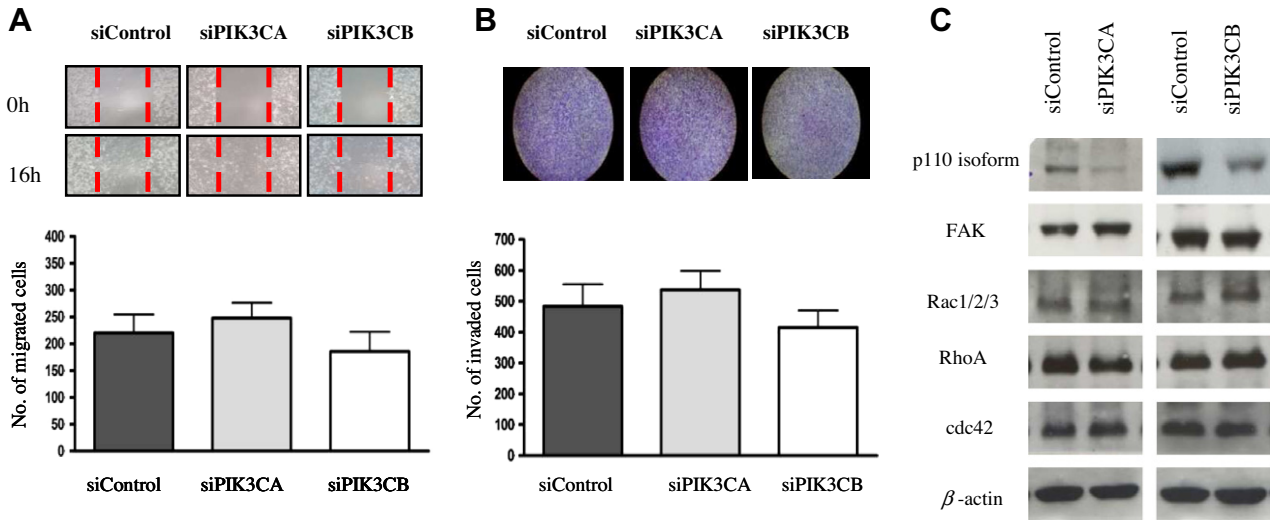

Fig. 4 – Cellular depletion of the catalytic PI3K isoforms p110α and p110β does not impair migration or invasion of U-87MG glioma cells *in vitro*. Cells were transfected with siPI3KCA, siPI3KCB, or siControl oligos. Thereafter, the number of cells undergoing (A) migration or (B) cell invasion was determined. (C) FAK and small GTPases expression in these cells exhibited no detectable changes upon p110α and p110β depletion.

glioma pathobiology. In contrast, pharmacological specific targeting of p110δ by two independent inhibitors resulted in significant reduction of cell migration, but not invasion (consistent with unchanged FAK and cdc42 levels). These data may indicate possible kinase-dependent and -independent roles of p110δ in glioma pathogenesis.

4. Discussion

In this study, we demonstrate for the first time that the catalytic PI3K subunit p110δ is expressed in a variety of human glioma tumour cell lines. Functionally, by targeting p110δ expression using siRNAs we demonstrate that p110δ expression is mandatory for efficient cell migration and invasion *in vitro* presumably through the regulation of FAK and cdc42 Rho GTPase expression. Moreover, p110δ function is obviously PI3K isoform specific and not compensated by p110α and p110β, which we detected in all glioma lines analysed, and which knockdown failed to influence glioma cell migration and invasion.

Malignant glioblastoma is the most common primary brain tumour in adults. Despite advanced therapeutic approaches being developed, the prognosis of GBM patients remains very poor.[18] Recent understanding of the molecular mechanisms in GBM pathogenesis has shed light on the 'target therapy' approach. One of these targets is the class I_A PI3K signalling pathway. However, given that PI3K signalling is a central and crucial controller of many normal cellular responses, complete inhibition of cellular PI3K function using broad range inhibitors is therapeutically not feasible.[23,24] Therefore, elucidation of specific roles of RTK-activated class I_A PI3K isoforms (i.e. p110α, p110β, and p110δ) is required in order to define a rationale for the therapeutic use of isoform-specific inhibitors. Towards this goal, substantial studies on the biochemical, structural properties and chemotypical diversities in different isoform-selective inhibitors of PI3K family have been performed. For example, IC87114, a preclinical p110δ-selective inhibitor, inhibits acute myeloid leukaemia cell proliferation and survival.[25] Another p110δ specific inhibitor, CAL-101, is the only inhibitor in clinical trial for haematological malignancies and management of allergic response.[26]

The PI3K isoform p110δ is linked to normal and pathophysiological immune cell functions including allergies and inflammatory responses.[27–29] However, different human cancer cell lines have been shown to express p110δ as well.[12–14] Overexpression of p110δ may play a major role in Akt activation, which in turn promotes cell proliferation, survival as well as cell migration in breast cancer,[12] neuroblastoma[14] and acute myeloid leukaemia cells.[30] This indicates that the unusual expression of p110δ in tumours may contribute to the malignant properties of cancer cells.[31] In agreement with these studies, we show that glioma cells express high levels of p110δ, and appear to play a crucial role in controlling glioma cell migration and invasion.

Glioblastoma multiforme is a highly infiltrative cancer and therefore, cell migration and invasion are particularly important processes in its progression. Both cell migration and invasion are complex processes which involve the coordination of several signal transduction pathways such as FAK and cytoskeletal signalling that allow cancer cells to remodel their local environment, migrate and invade surrounding tissues. Cell movement involves actin polymerisation, release of focal adhesions and production of extracellular proteases.[32,33] Our results indicate that the motility of glioma cells is potentially linked to specific signalling pathways involving p110δ, which regulates FAK and cytoskeletal-associated proteins. Interestingly, PI3K binding is required for FAK to promote cell migration, in which PI3K inhibitors were able to inhibit FAK-promoted migration in a dose-dependent manner.[34] FAK is a non-receptor tyrosine kinase protein that serves as a major

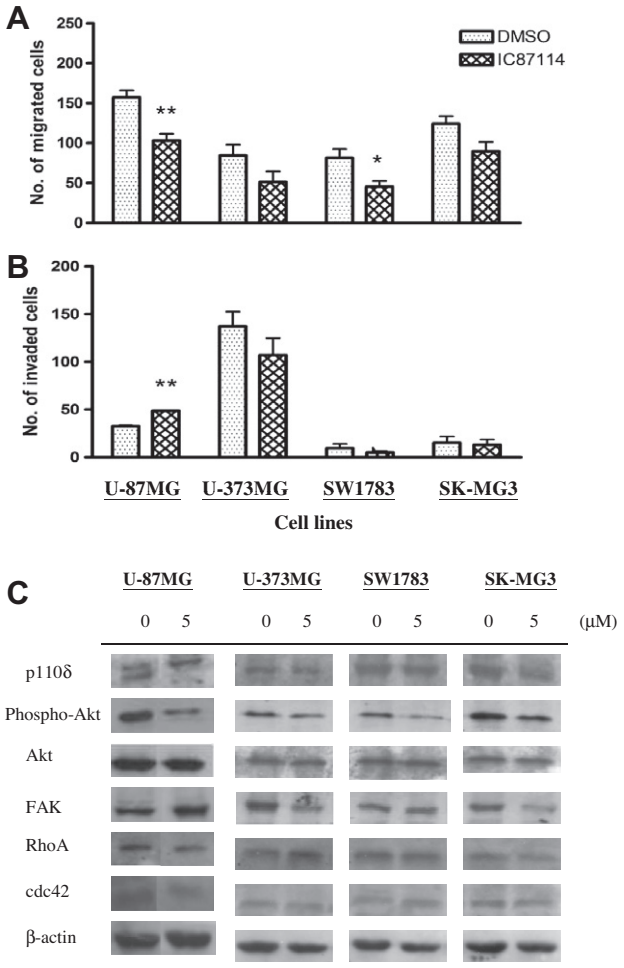

Fig. 5 – Pharmacological p110δ inhibition does impair glioma cell migration but not invasion *in vitro*. (A) Glioma cells treated with a p110δ specific inhibitor, IC87114, for 24 h generally showed significant reduction in migration (A) but had little or no effect on cell invasion (B) ($^{*}P < 0.05$, $^{**}P < 0.01$). (C) Phospho-Akt (Ser473) was substantially reduced upon pharmacological p110δ targeting, but the effect on FAK and small GTPases was not significant.

mediator of cell migration.[35] It functions as part of a cytoskeletal-associated network of intracellular signalling proteins that are activated by transmembrane integrin receptor clustering. Interestingly, FAK is expressed at elevated levels in GBM when compared with normal brain tissue.[36] Moreover, actin reorganisation occurs through FAK/PI3K activation in various human cancer cells, suggesting the association of FAK and PI3K regulates the cell motility process.[37] Within the Rho family of small GTPases, Rho, cdc42 and Rac facilitate the migratory and invasive behaviour of tumour cells by regulating actin and microtubule cytoskeleton organisation.[38] Most of the available data on the function of Rho family proteins in malignant glioma transformation are based on dominant-negative mutants and RNA interference-mediated depletion of these GTPases. Irrespectively, strong correlations between the expression of Rho GTPase proteins and the invasive behaviour of glioma cells have been reported.[39,40] In addition, radiation enhanced the invasiveness of primary glioblastoma cells through the activation of Rho signalling via PI3K.[41] Lastly, inhibiting PI3K pharmacologically also inhibits Rac1 and cdc42 activity in fibroblasts, epithelial cells and neutrophils.[42–44] All the above findings provide strong evidence that the regulation of FAK and Rho GTPase signalling is linked to PI3K. However, it is not clear which PI3K isoform is involved in this regulation, particularly in glioma cells. In the present study, p110δ is shown to be linked to the regulation of FAK and Rho GTPase signalling, because depletion of the p110δ isoform causes downregulation of FAK and cdc42 protein levels and hence likely signalling.

We also tested the p110δ specific and well characterised inhibitors IC87114 and CAL-101 on glioma cell migration and invasion. Comparable to p110δ knockdown, these inhibitors also reduced glioma cell migration. In contrast, inhibition of p110δ activity did not have any significant effect on glioma cell invasion. This suggests that on a putative molecular effector level the consequences of p110δ depletion and inhibition are different. Our data indicate that p110δ knockdown rather influences FAK and cdc42 downstream cascades with a direct impact on regulation of cell movement and invasion whereas pharmacological inhibition of p110δ leads to decrease in pAkt levels and presumably to Akt downstream cascades relating to cell migration only.[45,46] Although a specific role of the PI3K p110δ isoform in glioma cell migration and invasion has been identified in this study, detailed molecular mechanisms remain to be determined, including the molecular link of p110δ to the function of integrins/FAK, matrix metalloproteinases, and adhesion molecules. The data obtained in the present study nevertheless highlight the non-redundant role of the PI3K p110δ isoform in gliomagenesis and thus support the notion of developing isoform-specific p110δ inhibitors for the treatment of glioblastomas.

Clinically, temozolomide, a proautophagic cytotoxic drug, remains currently the key chemotherapeutic agent in the treatment of GBM. However, specific targeting of cell migration may include an option in the treatment of this tumour type. The results of this study support this notion and suggest that a siRNA approach as well as the use of PI3Kδ-specific inhibitors may be useful in downregulating gliomagenesis. In line with this, inhibitors of the downstream effectors of PI3Kδ, namely FAK and cdc42, may also be useful therapeutic targets. Overall, identification of anti-migratory and/or anti-invasion compounds, may lead to the development of novel treatment regimes such as combination therapy for more favourable prognostic benefits to glioblastoma patients.[47,48]

Role of the funding source

The funding source has no involvement in the study design, in the collection, analysis and interpretation of data, in the writing of the manuscript, and in the decision to submit the manuscript for publication.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2011.09.006.

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