Src homology domain-containing phosphatase 2 suppresses cellular senescence in glioblastoma

L-M Sturla*,1,2,4, PO Zinn1,2,4, K Ng2, M Nitta2,3, D Kozono2, CC Chen1,2,5 and EM Kasper1,5

1Department of Neurosurgery, Beth Israel Deaconess Medical Center, Boston, MA, USA; 2Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, MA, USA; 3Department of Neurosurgery, Tokyo Women’s Medical University, Tokyo, Japan

BACKGROUND: Epidermal growth factor receptor (EGFR) signalling is frequently altered during glioblastoma de novo pathogenesis. An important downstream modulator of this signal cascade is SHP2 (Src homology domain-containing phosphatase 2).

METHODS: We examined the The Cancer Genome Atlas (TCGA) database for SHP2 mutations. We also examined the expression of a further 191 phosphatases in the TCGA database and used principal component and comparative marker analysis available from the Broad Institute to recapitulate the TCGA-defined subgroups and identify the specific phosphatases defining each subgroup. We identified five siRNAs from two independent commercial sources that were reported by the vendor to be pre-optimised in their specificity of SHP2 silencing. The specificity and physiological effects of these siRNAs were tested using an in vitro glioma model.

RESULTS: TCGA data demonstrate SHP2 to be mutated in 2% of the glioblastoma multififorme’s studied. Both mutations identified in this study are likely to be activating mutations. We found that the four subgroups of GBM as defined by TCGA differ significantly with regard to the expression level of specific phosphatases as revealed by comparative marker analysis. Surprisingly, the four subgroups can be defined solely on the basis of phosphatase expression level by principal component analysis. This result suggests that critical phosphatases are responsible for the modulation of specific molecular pathways within each subgroup. Src homology domain-containing phosphatase 2 constitutes one of the 12 phosphatases that define the classical subgroup. We confirmed the biological significance by siRNA knockdown of SHP2. All five siRNAs tested reduced SHP2 expression by 70–100% and reduced glioblastoma cell line growth by up to 80%. Profiling the established molecular targets of SHP2 (ERK1/2 and STAT3) confirmed specificity of these siRNAs. The loss of cell viability induced by SHP2 silencing could not be explained by a significant increase in apoptosis alone as demonstrated by terminal deoxynucleotidyl transferase-mediated nick-end labelling and propidium iodide staining. Src homology domain-containing phosphatase 2 silencing, however, did induce an increase in β-galactosidase staining. Propidium iodide staining also showed that SHP2 silencing increases the population of glioblastoma cells in the G1 phase of the cell cycle and reduces the population of such cells in the G2/M- and S-phase.

CONCLUSION: Src homology domain-containing phosphatase 2 promotes the growth of glioblastoma cells by suppression of cellular senescence, a phenomenon not described previously. Selective inhibitors of SHP2 are commercially available and may be considered as a strategy for glioblastoma therapy.

Keywords: glioblastoma; phosphatases; SHP2; senescence

Glioblastoma multiforme (GBM) is the most common type of malignant primary brain tumour in adults. Around 30 000 new cases are diagnosed every year in the United States and Europe (CBTRUS, 2008). The prognosis is dismal, and despite treatment with the standard of care regimen involving surgery, radiation and chemotherapy, median survival remains below 15 months (Stupp et al, 2005), and there is a clear need for improved therapeutic approaches. There has, however, been substantial progress in the understanding of molecular cancer subgroups (Verhaak et al, 2010), pathways involved in gliomagenesis and disease progression (Furnari et al, 2007; Network CGAR, 2008; Parsons et al, 2008; Yan et al, 2009; Verhaak et al, 2010) These efforts to understand the underlying molecular biology of the disease is now paving the way for the development of targeted therapeutics.

Epidermal growth factor receptor (EGFR) is overexpressed in a variety of human tumours including GBM, where it has been linked to radiation resistance and poor prognosis. A number of researchers, including us, have shown activation of EGFR to result in cytoprotective and proliferative downstream signalling (Schmidt-Ullrich et al, 2003; Sturla et al, 2005). Studies of receptor tyrosine kinases (RTKs), including EGFR, demonstrate that the overall phosphorylation state is a net result of RTK and protein tyrosine phosphatase (PTP) activities (Reynolds et al, 2003). As the catalytic activity of PTP’s can be 1000-fold greater than that of
kinases (Haque et al., 1995; Wang et al., 2010), perturbation of activity may have a significantly more profound effect on signal propagation than that of kinases. Most targeting strategies for RTKs emphasise the kinase activity (Shimizu et al., 2008). In autocrine-regulated tumour cells, the Trk kinase activity is always ‘on’, and thus net RTK activity will be mostly regulated by PTP activity. This suggests that the greatest therapeutic gain may be achieved by targeting the counteracting PTP.

Src homology domain-containing phosphatase 2 (SHP2) (PTPN11) is a non-receptor PTP, which regulates several of the RTK pathways known to be overexpressed in glioblastoma, including EGFR, FGFR and PDGFR (Grossmann et al., 2010). Perhaps, the most well-studied role of SHP2 is that in the modulation of EGFR phosphorylation, the RTK most widely overexpressed in GBM (Bredel et al., 2010). Here SHP2 has been shown to both antagonise and potentiate the action of its target PTK’s and was the first phosphatase described as oncogenic (Bentires-Alj et al., 2004). More than 58 different SHP2 mutations have been identified in various tumours and 18 mutations in Noonan and Leopard syndromes, where patients exhibit disruption of normal cell proliferation and migration during development (Bentires-Alj et al., 2004). In its basal state, SHP2 activity is suppressed by intramolecular interactions between residues in the ‘backside loop’ of the N-terminal SH2 domain and the catalytic surface of the PTP domain (Hoé et al., 1998). The mutations have been found to cluster mostly in the N-SH2 and PTP domain interface of the protein and are therefore predicted to be activating mutations – suggesting a positive role for SHP2 in tumourigenesis.

As mutations in SHP2 have been identified in a variety of solid tumours and this phosphatase is an important regulator of multiple RTK’s involved in the aetiology of GBM, we decided to examine this phosphatase closely in both established GBM cell lines and the The Cancer Genome Atlas (TCGA) human tissue database. We used both TCGA mutation and expression data to confirm the presence of SHP2 mutations in human GBM and establish a potential role for this phosphatase in the classical, RTK-driven subgroup. We also used an siRNA approach to examine the effects of SHP2 knockdown on cell viability in established GBM cell lines in vitro.

MATERIALS AND METHODS

TCGA analysis

Median expression data for 207 GBM samples from two different microarray platforms combined was downloaded from the TCGA data portal. Sample data were rearranged to group tumours into the four subclasses defined by TCGA (Verhaak et al., 2010). The resulting file was converted to a gct file compatible with the GenePattern software available from the Broad Institute (http://www.broadinstitute.org/). Files were generated from this master file containing either the 189 phosphatase genes available on the microarray platforms or the 189 phosphatase genes combined with the 816 remaining genes allowing a comparison to the whole genome. Principal component analysis (PCA) was performed on both the phosphatase and whole genome data sets using the gene pattern software.

The GenePattern software was used to perform K nearest-neighbour analysis (Sun et al., 2011) using leave-one-out cross-validation (KNN X-validation). This class prediction analysis was used to determine how accurately the samples could be grouped into their various classes using only phosphatase expression as compared to the whole genome.

Gene pattern comparative marker analysis (CMA) was used to identify significant markers for each group using only phosphatase genes, phosphatase and kinase genes combined or the entire gene set. Marker phosphatases were then fed into both the pathway interaction database (http://pid.nci.nih.gov/) and ingenuity pathway analysis (http://www.ingenuity.com/) to look for enrichment of pathways in each group.

Cell lines and tissue culture

Established glioblastoma cell lines, U87 and A172, were obtained from the ATCC and maintained in Dulbecco’s modified Eagle’s medium high glucose with Penstrep and 15% FBS (Invitrogen, Carlsbad, CA, USA) at 37 °C, 5% CO₂.

Transfection with siRNA

Cells were cultured to a confluency of 30% before siRNA transfection. Following this siRNA was transfected into cells using Lipofectamine RNAiMAX (Invitrogen). Transfection was carried out according to the manufacturer’s instructions using a final concentration of 10 nm siRNA. Transfection efficiency was assessed using a fluorescent non-targeting siRNA (Allstars Negative siRNA Alexafluor488; Qiagen, Valencia, CA, USA). The non-targeting control siRNA (Allstars Negative siRNA) and SHP2-specific siRNAs Hs_PTPN11_7 (Q1; SI02225909) and Hs_PTPN11_6 (Q2; SI02225902) were also purchased from Qiagen. Stealth RNAi-negative control low GC duplex and high GC duplex and the SHP2-specific siRNA PTPN11HSS108832 (I1), PTPN11HSS108833 (I2) and PTPN11HSS108834 (I3) were purchased from Invitrogen.

Western blotting and antibodies

Cells were rinsed with ice-cold phosphate-buffered saline (PBS) and snap-frozen on dry ice at the appropriate times following transfection. Cells were scraped into a denaturing cell extraction buffer (Invitrogen) containing a protease inhibitor cocktail (Pierce, Rockford, IL, USA) and 100 μg mL⁻¹ PMSF. Lysates were incubated for 30 min on ice and passed five times through a 20-gauge needle and syringe. Samples were centrifuged at 14,000 g for 15 min at 4 °C, and supernatant protein concentrations were determined by the Bradford assay (Biorad, Hercules, CA, USA). For whole-cell lysates, 5 × loading buffer (50 mM NaPO₄, 5% SDS, 0.25% bromophenol blue, 12.5% 2-mercaptoethanol and 10% glycerol) was added to lysates to achieve 1 × . Equal amounts of protein were fractionated on SDS/10% polyacrylamide gels and protein transferred electrophoretically onto nitrocellulose membranes. Membranes were probed with the appropriate primary and secondary antibodies. Blots were analysed by chemiluminescence detection (Supersignal West Pico; Pierce). The SHP2 antibody was used at a dilution of 1:500; phospho- and total STAT3 dilution was 1:500; and media were changed every 3 days to avoid assay error due to media evaporation. At 6 and 10 days post-transfection, alamar blue (Invitrogen) was added at a concentration of 10% per well and cells incubated for 3 h at 37 °C, 5% CO₂. Colorimetric change
(fluorescence) was assessed using a Biorad plate reader, Ex 570 nm and Em 585 nm.

β-Galactosidase assay

Cells cultured and transfected for viability assay were seeded in triplicates at a density of 1000 cells per well in 24-well plates at the time of re-seeding for viability assay. Cells were incubated at 37 °C for 4 days following reseeding and stained for β-galactosidase, using a β-galactosidase staining kit according to the manufacturer’s instructions (Cell Signaling Technology). Plates were viewed and photographed using a Zeiss inverted microscope, ×20 magnification.

TUNEL and cell cycle analysis

Cells were rinsed in PBS and assessed for DNA degradation by incubation with RNAse (100 μg ml⁻¹; Invitrogen) for 30 min before propidium iodide labelling (TUNEL) according to the manufacturer’s instructions (Apoptag; Intergen, Burlington, MA, USA). Analysis of cell cycle labeling (TUNEL) according to the manufacturer’s instructions (Cell Signaling Technology). Plates were viewed and photographed using a Zeiss inverted microscope, ×20 magnification.

RESULTS

GBM harbours SHP2 mutations likely to be activating mutations

The TCGA identified four mutations of SHP2 in tumour normal matched GBM samples. Two of these mutations were validated and found to be present in tumour samples only (Figure 1). The first mutation is a single base mutation, resulting in a glutamic acid to lysine change at position 69. This amino acid is situated within the N-terminal SH2 domain and forms a stabilising hydrogen bond with aspartic acid 281 in the PTP domain. The mutation, originally identified in the neuroblastoma, was copied in vitro and found to have a 16-fold higher phosphatase activity than wild-type SHP2 (Bentires-Alj et al, 2004). The N-SH2 domain sits in the catalytic site of the PTP domain inhibiting SHP2 phosphatase activity. Not surprisingly, mutations in the N-SH2 domain have been found to significantly increase SHP2 phosphatase activity (Bentires-Alj et al, 2004).

The second mutation results in an isoleucine to methionine substitution at position 282 within the PTP domain. This is adjacent to the 281 aspartic acid residue involved in the hydrogen bond shown to stabilise the inactive conformation of SHP2. Although this mutation has not previously been described, its location suggests that it could also be an activating mutation.

The third mutation results in a leucine to histidine substitution at position 553 within the c-terminal domain. This is a threonine to methionine substitution at position 553 within the c-terminal domain. However, these final two mutations were not validated as tumour-specific.

SHP2 is a marker of the classical subgroup of GBM

We found that the four subgroups of GBM, as defined by TCGA, differ significantly with regard to the expression level of specific phosphatases. The four subgroups can be defined solely on the basis of phosphatase expression by PCA (Figure 2A).

The gene pattern software was used to perform K nearest-neighbor analysis using leave-one-out cross-validation. This class prediction analysis was used to determine how accurately the samples could be grouped into their various classes using only phosphatase expression as compared to the combined phosphatase/kinase or whole genome. K nearest-neighbour cross-validation using only phosphatase gene expression accurately predicted the sample class for 64.5 ± 6.5% of the samples when analysis was performed on both the original and validation data sets. This compares to 75 ± 0% when using the whole genome (Figure 2B). When the accuracy of prediction is broken down by class, however, the phosphatase-only gene set accurately predicts

**Figure 1** The Cancer Genome Atlas mutation data reveal potential activating mutations of SHP2 in glioblastoma. The TCGA mutation database was searched for all mutations of SHP2. Only those mutations validated in tumour vs normal are shown here. (A) Structural representation of SHP2 showing the mutations found in GBM, which cluster in the N-SH2 and PTP domain interfaces. (B) Schematic representation of SHP2. (C) Mutation data for validated SHP2 mutations identified by TCGA and validated against paired normal tissue.
Inhibition of SHP2 induces glioblastoma senescence
L-M Sturla et al

Phosphatases and Genomics 1238

The sample class for 74 ± 7% of the classical samples compared to 73 ± 5% using the whole genome. There was no significant difference between percentage correctly assigned samples determined using phosphatase expression only and that determined using the whole genome (unpaired \( t \)-test, \( P = 0.17–0.92 \)).

Comparative marker analysis identified SHP2 as one of the 12 phosphatases that define the classical subgroup (Figure 2C). In all, 12 phosphatases were found to be significantly associated with classical GBM, with SHP2 being sixth on the list with a \( P \)-value < 0.0001. When CMA was used with the phosphatase and kinase genes combined, SHP2 fell to the 40th position with a \( P \)-value of 0.032. Src homology domain-containing phosphatase 2 therefore holds its significance as a classical subgroup-defining phosphatase even in the context of the entire gene set. When this analysis was repeated using a validation data set available from TCGA, SHP2 remained as a significant marker of the classical subgroup (Figure 2C).

Figure 2  Analysis of TCGA profiling data reveal potential role of SHP2 in defining the classical subgroup of GBM. (A) Principal component analysis of TCGA GBM data using 191 phosphatase genes or the whole genome. (B) K nearest-neighbour analysis using leave-one-out cross-validation (KNN X-validation) and the most significant 10 features (most differentially expressed genes) were used to determine how accurately the samples could be grouped into their various classes using only phosphatase expression vs the whole genome. Data are shown for both test and validation data sets as the mean percentage correctly assigned samples ± s.e.m. There was no significant difference between the percentage of correctly assigned samples determined using phosphatase expression only and the comparative value as determined using the whole genome (unpaired \( t \)-test, \( P = 0.17–0.92 \)). (C) Comparative marker analysis identified most significant genes that define the classical subgroup of GBM using phosphatase genes only, phosphatase and kinase genes combined or the whole gene expression profile. Validation data results are shown within square parenthesis [ ].

SHP2 siRNA decreases GBM cell viability without a significant increase in apoptosis

All five siRNAs tested reduced SHP2 expression by 70–100% as compared to the non-targeting siRNA or a mock-transfected control from 36 h post-transfection (Figure 3). Equivalence of protein loading was confirmed by actin staining. Profiling the phosphorylation status of established molecular targets of SHP2 (ERK1/2 and STAT3) (Agazie and Hayman, 2003; Zhang et al, 2009) using the whole genome (unpaired \( t \)-test, \( P = 0.032 \)).

Figure 3  Specificity of SHP2 siRNA was confirmed by western blot of well-characterised downstream targets of SHP2, ERK1/2 and STAT3. (A) SHP2 dephosphorylates RAS-GAP binding sites on RTK’s, resulting in enhanced RAS activity and consequent MAPK phosphorylation. Src homology domain-containing phosphatase 2 negatively regulates RAS-GAP binding sites on RTK’s. (B) Specificity of SHP2-specific siRNA was shown by determination of the phosphorylation status of the SHP2 targets ERK1/2 and STAT3 3–6 days following transfection with 10 nM siRNA.
confirmed specificity of these siRNAs. Src homology domain-containing phosphatase 2 has previously been shown to enhance Ras activation by dephosphorylating EGFR tyrosine 992, responsible for the translocation of Ras GAP to the plasma membrane, where it inhibits Ras activity (Agazie and Hayman, 2003). Knockdown of SHP2 by SHP2-specific siRNA resulted in a decrease in ERK1/2 phosphorylation without a significant change in total ERK1/2 levels as shown by western blot. In contrast, SHP2 has been shown to have an inhibitory effect on JAK-STAT signalling (Zhang et al., 2009). In accordance with the established effect of SHP2 on STAT3, knockdown of SHP2 siRNA resulted in an increase in phospho-STAT3 without a significant increase in total STAT3 levels (Figure 3).

The viability of U87 and A172 cells transfected with non-targeting siRNA was not significantly different to that of untransfected or mock-transfected cells (data not shown). Viability of U87 and A172 cells transfected with SHP2-specific siRNA reduced glioblastoma cell line growth by up to 80%, as shown using an alamar blue assay (Figure 4). Five commercially available SHP2-specific siRNAs were tested. Of these, one siRNA had a much greater effect on cell viability than the others, siRNA I2, and was eliminated from further study in case of off-target toxic effects. Viability of U87 cells was reduced by 60–75% by the four remaining siRNAs (P < 0.0001). A172 cell viability was reduced by up to 60% (P < 0.005).

Reduced cell viability could not be accounted for by enhanced apoptosis as shown by TUNEL. The classical DNA degradation associated with apoptosis was assessed by TUNEL in non-transfected, non-targeting siRNA-transfected and SHP2-specific siRNA-transfected cells. Less than 1% of the control cell populations were shown to be apoptotic as determined by TUNEL positivity (Figure 5). U87 cells transfected with either Q1 or Q2 SHP2-specific siRNA exhibited TUNEL positivity in 1% and 2% of the cell population, while I1 and I3 showed 5% and 3%, respectively (Figure 5A). Although the percentage of apoptotic cells was higher in SHP2 siRNA-transfected cells as compared to the non-transfected and non-targeting siRNA controls, this was not high enough to account for the loss of cell viability observed. Similarly in A172 cells, only 1% of the cell population was TUNEL positive. Although this percentage increased to 10%, 13% and 11% in Q1, I1 and I3 SHP2-specific siRNA-transfected cells, respectively, this was not high enough to explain reduced cell viability (Figure 5B).

**SHP2 siRNA induces cellular senescence**

Both U87 and A172 cells transfected with SHP2-specific siRNA showed significant morphological changes. Cells showed enlarged and flattened or elongated morphology consistent with that seen in senescent cells (Figure 6). Although similar morphological changes were noted in U87 cells (Zhan et al., 2009), cellular senescence was neither studied nor suggested as a mechanism.

As we saw a loss in cell viability without a significant increase in cellular apoptosis alongside morphological changes consistent with senescence, we further analysed the effects of SHP2 knockdown on cellular senescence. Cells with knockdown of SHP2 exhibited cellular enlargement and elongation classically associated with senescence (Figure 6). β-Galactosidase staining at pH 6.0 is also a classical marker of cellular senescence (Figure 6). Src homology domain-containing phosphatase 2 knockdown induced a significant increase in both intensity of β-galactosidase staining and number of β-galactosidase-positive cells in both U87 (Figure 6A) and A172 cells (Figure 6B) from 4 days post-transfection. Cells with >15% positivity as determined using the Imagej software (NIH) were considered positive for quantitation. Two fields per replicate and a total of 2000 cells were counted. Knockdown of SHP2 induced a highly significant increase in the percentage of β-galactosidase-positive cells in both U87 and A172 cells as compared to a control siRNA or mock-transfected control (Figure 6C). Cell populations transfected with SHP2 siRNA demonstrated 60–90% β-galactosidase positivity as compared to 20–40% β-galactosidase positivity in cells transfected with the negative control siRNA (P < 0.0001). An increase in p53 protein level, as determined by western blot, was also observed in the majority of SHP2 knockdown cells. This is consistent with the increase in p53 known to precede cellular senescence (Quick and Gewirtz, 2006). The increase in p53 level was inconsistent in Figure 6B (lane 5) due to limited sample. The actin loading control is also reduced in this lane. Owing to the drastic effect of SHP2 knockdown on cell viability, it is not always possible to get sufficient protein for western blot following viability and senescence assays.

Propidium iodide staining was also used to observe cell populations in the various phases of the cell cycle. Senescent cells have previously been shown to arrest in the late G1 phase of the cell cycle, and consistent with this phenomenon, we found an increase in the percentage of cells in the G1 phase of the cell cycle in SHP2 knockdown cells (data not shown).

**DISCUSSION**

Our analysis of TCGA data showed that phosphatase expression alone can be used to recapitulate the subdivision of GBM into four subgroups as identified by TCGA using the whole genome-derived
840 gene identifier (Verhaak et al., 2010), suggesting that phosphatases play an important role in the underlying biology of GBM. As kinases are far more numerous than phosphatases, one phosphatase must target multiple kinases. As such, targeting phosphatases may prove more effective in tumours, such as GBM, with co-activation of multiple RTK pathways, which respond poorly to kinase inhibitor monotherapy.

We identified activating mutations of the putative oncogenic phosphatase, SHP2, in approximately 2% of the tumours analysed by TCGA. Both of the mutations that passed validation in the TCGA data set could be considered activating mutations based on their location within the N-SH2/PTP domain interface. The first mutation, E69K, has been well studied and is known to have 16-fold higher phosphatase activity than wild-type SHP2 (Bentires-Alj et al., 2004), although a more recent study by the same group determined that the tumourigenic potential of SHP2 mutation does not necessarily correlate with PTP activity (Keilhack et al., 2005). With respect to the second mutation,
Figure 6  SHP2-specific siRNA induces senescence in U87 and A172 cells. Senescence was determined by the classic morphological changes, including enlarged and flattened or elongated morphology and β-galactosidase staining in (A) U87 and (B) A172 cells transfected with 10 nM control or SHP2-specific siRNA 4 days following transfection. Western blot was used to determine p53 level. Images were captured at a magnification of × 20. (C) Cells with > 15% positivity as determined using the ImageJ software (NIH) were considered positive for quantitation. Two fields per replicate and a total of 2000 cells were counted. Data are shown as percentage-positive cells ± s.e.m.
we predict this to be an activating mutation due to its location adjacent to a hydrogen bond known to stabilise the inactive conformation.

We also considered the expression profile of phosphatases in the four GBM subgroups described by TCGA. Comparative marker analysis identifying the phosphatases most significantly associated with each group clearly identified SHP2 as an important marker of the classical subgroup of GBM and remained a significant marker even when considered alongside kinases or the whole genome. Considering the well-documented role for SHP2 in the regulation of EGFR phosphorylation, it is not too surprising that the classical subgroup of glioblastoma, as defined by TCGA, was also the group found to have deregulation of EGFR signalling. High-level EGFR amplification was observed in 97% of this subtype along with 70% of the EGFRvIII mutations (Verhaak et al., 2010). Platelet-derived growth factor subunit A, also overexpressed in this group, has been shown to enhance EGFR signalling through heterodimerisation of PDGFR and EGFR (Milenkovic et al., 2003).

SHP homology domain-containing phosphatase 2 is well known to extend the half-life of active RAS and increase ERK1/2 signalling downstream of a variety of RTKs, including EGFR, PDGFR and FGFR, all of which have been found to be overexpressed in GBM (Gianninni et al., 2005).

The classical subgroup of glioblastoma, as defined by TCGA, was found to have high expression of EGFR and PDGFR. Platelet-derived growth factor subunit A has been shown to enhance EGFR signalling through heterodimerisation of PDGFR and EGFR (Milenkovic et al., 2003). High-level EGFR amplification was also observed in 97% of this subtype along with five of seven EGFRvIII mutations (Verhaak et al., 2010). Given that SHP2 has been shown by several groups to regulate EGFR activity (Wu et al., 2000; Grossmann et al., 2010), it is not surprising that we also found SHP2 to be a significant marker of the classical subgroup of GBM.

Given the association of SHP2 expression with the RTK-driven ‘classical’ subgroup of GBM and the identification of potential activating mutations in GBM samples, it is not surprising that knockdown of SHP2 with SHP2-targeting siRNA resulted in a loss of cell viability. This loss in viability, however, was not associated with a significant increase in apoptosis. We did see striking morphological changes in the cells with significant knockdown of SHP2. These changes were similar to those described as classical cellular senescence. Although the morphological changes observed in our study were noted in a previous study, no connection to cellular senescence was made (Zhan et al., 2009). We found various markers of senescence when we knocked down SHP2 expression in GBM cell lines, suggesting that SHP2 protects the cells from senescence. It is therefore possible that the presence of activating mutations of SHP2 in GBM allows tumour progression by protecting cells from senescence and apoptosis. The mechanism of action is unknown at present, but two strong possibilities exist. Src homology domain-containing phosphatase 2 is well known to enhance the half-life of active RAS and as such it is possible that the knockdown of SHP2 activity and its consequent effects on RAS activity are responsible for the senescence that we observe in GBM cell lines. We confirmed a reduction in the MAPIs, ERK1/2, downstream of RAS in cells with reduced SHP2 protein levels. Although RAS is an important mediator of glioblastoma tumourigenesis, its loss is not typically associated with the induction of cellular senescence. In fact, there are several reports of induction of senescence by overexpression of active RAS, a phenomenon known as oncogene-induced senescence (Rai et al., 2010; Kosar et al., 2011). Previous studies have also shown SHP2-activating mutations to be mutually exclusive to mutations such as NF1 loss, which leads to an increase in active RAS (Holzel et al., 2010).

SHP homology domain-containing phosphatase 2 has been shown, however, to regulate hTERT localisation (Jakob et al., 2008). The human telomerase catalytic subunit – when localised to the nucleus – protects cells against cellular senescence. Human telomerase reverse transcriptase alone has been found to immortalise normal human astrocytes (Sonoda et al., 2001) with the addition of SV40 T-Ag and active RAS (H-ras), allowing maximum tumourigenicity as determined by anchorage-independ-ent growth and formation of tumours in nude mice (Rich et al., 2001). This is consistent with the finding that most grade III gliomas express telomerase. Its reactivation is associated with non-malignant grade II to malignant grade III conversion (Kim et al., 1994; Sano et al., 1998). A study by Jakob et al. (2008) showed overexpression of SHP2 to block oxidative stress-induced nuclear export of hTERT. As a consequence, hTERT is retained in the nucleus, resulting in resistance to cellular senescence and apoptosis. Preliminary data using an hTERT antibody and IHC (data not shown) suggest that knockdown of SHP2 expression using siRNA reduces nuclear hTERT staining in U87 cells. More work is required to confirm these data and to determine the exact mechanism by which SHP2 suppresses cellular senescence in glioblastoma, but it appears likely that it plays a crucial role in the viability of these cells. As the selectivity of commercially available SHP2 inhibitors is improved, they should be considered a potential strategy for glioblastoma therapy.

REFERENCES

Agazie YM, Hayman MJ (2003) Molecular mechanism for a role of SHP2 in epidermal growth factor receptor signaling. Mol Cell Biol 23: 7875–7886
Bentires-Alj M, Paez JG, David FS, Keilhack H, Halmos B, Naoki K, Bredel M, Scholtens DM, Yadav AK, Alvarez AA, Renfrow JJ, Chandler JP, Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C, Chin L, DePinho RA, Cavenee WK (2007) Malignant astrocytic gloma: genetics, biology, and paths to treatment. Genes Dev 21: 2683 – 2710
Gianninni C, Sarkaria JN, Saito A, Uhm JH, Galanis E, Carlson BL, Schroeder MA, James CD (2005) Patient tumor EGFR and PDGFR gene amplifications retained in an invasive intracranial xenograft model of glioblastoma multiforme. Neuro-oncology 7: 164 – 176
Grossmann KS, Rosario M, Birchmeier C, Birchmeier W (2010) The tyrosine phosphatase Shp2 in development and cancer. Adv Cancer Res 106: 53 – 89
Haque SJ, Flati V, Deb A, Williams BR (1995) Roles of protein-tyrosine phosphatases in Stat1 alpha-mediated cell signaling. J Biol Chem 270: 2579 – 25714
Hof P, Pluskey S, Dhe-Paganon S, Eck MJ, Shoeldson SE (1998) Crystal structure of the tyrosine phosphatase SHP-2. Cell 92: 441–450
Holzel M, Huang S, Koster J, Ora I, Lakeman A, Caron H, Nijkamp W, Xie J, Callens T, Asgharzadeh S, Seeger RC, Messiaen L, Versteeg R, Bernards R (2010) NF1 is a tumor suppressor in neuroblastoma that determines retinoic acid response and disease outcome. Cell 142: 218 – 229
Jakob S, Schroder P, Lucosz M, Spyridopoulos I, Alschmeid J, Haendler J (2008) Nuclear protein tyrosine phosphatase Shp-2 is one important...
negative regulator of nuclear export of telomerase reverse transcriptase. J Biol Chem 283: 33155 – 33161
Keilhack H, David FS, McGregor M, Cantley LC, Neel BG (2005) Diverse biochemical properties of Shp2 mutants: implications for disease phenotypes. J Biol Chem 280(35): 30984 – 30993
Kim NW, Piatsyzk MA, Prowse KR, Harley CB, West MD, Ho PL, Covilli GM, Wright WE, Weinrich SL, Shay JW (1994) Specific association of human telomerase activity with immortal cells and cancer. Science 266: 2011 – 2015
Kosar M, Bartkova J, Hubackova S, Hodny Z, Lukas J, Bartek J (2011) Senescence-associated heterochromatin foci are dispensable for cellular senescence, occur in a cell type- and insult-dependent manner, and follow expression of p16 (ink4a). Cell Cycle 10: 457 – 468
Milenkovic I, Weick M, Wiedemann P, Reichenbach A, Bringga M (2003) P2Y receptor-mediated stimulation of Muller glial cell DNA synthesis: dependence on EGF and PDGF receptor transactivation. Invest Ophthalmol Vis Sci 44: 1211 – 1220
Network CGAR (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455: 1061 – 1068
Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz Jr LA, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Parmigiani G, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kim KR (2008) An integrated genomic analysis of human glioblastoma multiforme. Science (New York, NY) 321: 1070 – 1075
Quick QA, Gewirtz DA (2006) An accelerated senescence response to iP, Youn J, Burton DG, Gribaldi MG, Onder TT, Weinberg RA (2010) Induction of senescence, apoptosis, and DNA damage response (DDR) in glioblastoma cells by inhibition of the tyrosine phosphatase SHP2. Cancer Res 70: 5182 – 5191
Sano T, Asai A, Mishima K, Fujimaki T, Kirino T (1998) Telomerase activity in human glioblastoma cells and core pathways. Cancer Res 58: 5456 – 5460
Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352: 987 – 996
Sturla LM, Amorino G, Alexander MS, Mikkelson RB, Valerie K, Schmidt-Ullrich RK (2005) Requirement of Tyr-992 and Tyr-1173 in phosphorylation of the epidermal growth factor receptor by ionizing radiation and modulation by SHP2. J Biol Chem 280: 14597 – 14604
Sun X, Mei S, Tao H, Wang G, Su L, Jiang S, Deng C, Xiong Y, Li F (2011) Microarray profiling for differential gene expression in PMSC-hCG stimulated preovulatory ovarian follicles of Chinese Taihu and Large White sows. BMC Genom 12: 111
Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, Miller CR, Ding L, Golub T, Mesirov JP, Alexe G, Lawrence M, O’Kelly M, Tamayo P, Weir BA, Gabriel S, Winckler W, Gupta S, Jakkula L, Feller HS, Hodgson JG, James CD, Sarkaria JN, Brennan C, Kahn A, Spellman PT, Wilson RK, Speed TP, Gray JW, Meyerson M, Getz G, Perou CM, Hayes DN (2010) Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 17: 98 – 110
Wang V, Davis DA, Veeranna RP, Haque M, Yarchoan R (2010) Characterization of the activation of protein tyrosine phosphatase, receptor-type, Z polypeptide 1 (PTPRZ1) by hypoxia inducible factor-2 alpha. PLOs One 5: e9641
Wu CJ, Chen Z, Ulrich R, Greene MI, O’Rourke DM (2000) Inhibition of EGFR-mediated phosphoinositide-3-OH kinase (PI3-K) signaling and glioblastoma phenotype by signal-regulatory proteins (SIRPs). Oncogene 19: 3999 – 4010
Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, Friedman H, Friedman A, Reardon D, Herndon J, Kinzler KW, Velculescu VE, Vogelstein B, Bigner DD (2009) IDH1 and IDH2 mutations in gliomas. N Engl J Med 360: 765 – 773
Zhan Y, Counelis GJ, O’Rourke DM (2009) The protein tyrosine phosphatase SHP-2 is required for EGFRVIII oncogenic transformation in human glioblastoma cells. Exp Cell Res 315: 2343 – 2357
Zhang W, Chan RJ, Chen H, Yang Z, He Y, Zhang X, Luo Y, Yin F, Moh A, Miller LC, Payne RM, Zhang ZY, Fu XT, Shou W (2009) Negative regulation of Stat3 by activating PTPN11 mutants contributes to the pathogenesis of Noonan syndrome and juvenile myelomonocytic leukemia. J Biol Chem 284: 22353 – 22363

This work is published under the standard license to publish agreement. After 12 months the work will become freely available and the license terms will switch to a Creative Commons Attribution-NonCommercial-Share Alike 3.0 Unported License.