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Priority Report

Cancer Research

Integrin α5β1 Plays a Critical Role in Resistance to Temozolomide by Interfering with the p53 Pathway in High-Grade Glioma

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Abstract

Integrins play a role in the resistance of advanced cancers to radiotherapy and chemotherapy. In this study, we show that high expression of the α5 integrin subunit compromises temozolomide-induced tumor suppressor p53 activity in human glioblastoma cells. We found that depletion of the α5 integrin subunit increased p53 activity and temozolomide sensitivity. However, when cells were treated with the p53 activator nutlin-3a, the protective effect of α5 integrin on p53 activation and cell survival was lost. In a functional p53 background, nutlin-3a down-regulated the α5 integrin subunit, thereby increasing the cytotoxic effect of temozolomide. Clinically, α5β1 integrin expression was associated with a more aggressive phenotype in brain tumors, and high α5 integrin gene expression was associated with decreased survival of patients with high-grade glioma. Taken together, our findings indicate that negative cross-talk between α5β1 integrin and p53 supports glioma resistance to temozolomide, providing preclinical proof-of-concept that α5β1 integrin represents a therapeutic target for high-grade brain tumors. Direct activation of p53 may remain a therapeutic option in the subset of patients with high-grade gliomas that express both functional p53 and a high level of α5β1 integrin. *Cancer Res; 72(14); 3463–70.* ©*2012 AACR.*

Introduction

Glioblastoma multiforme (GBM) are the most aggressive brain tumors and remain a challenge for oncologists. New therapies are urgently needed. Gene expression profiling of high-grade glioma revealed that genes of extracellular matrix components and their regulators are often affected in the patients. Fibronectin is overexpressed in glioblastoma versus normal brain (1) and belongs to the cluster of genes associated with a more malignant phenotype (2, 3). It has recently been shown that fibronectin knockdown delays tumor growth in a mouse glioma model (4). The α5β1 integrin is a fibronectin receptor that was recently shown to have an important role in tumor progression (5), metastasis (6), and/or resistance to therapies (7) in lung, ovarian, and breast cancer, respectively. Few works addressed directly the issue of α5β1 integrin in glioma. Through the use of non-peptidic α5β1 integrin antagonists and GBM cell lines, we previously showed that α5β1 integrin may be a therapeutic target for these tumors (8, 9) and that concomitant addition of α5β1 antagonists sensitizes p53 wild-type (p53-wt) glioma cells to chemotherapeutic drugs (10). The presence of p53 mutations in high-grade glioma varied across GBM subtypes with 0%, 21%, 32%, and 54% in classical, neural, mesenchymal, and proneural subtypes, respectively (11). There is increasing evidence that gliomas harboring a p53-wt resist to therapies through inhibitory pathways upstream of p53. Nutlin-3 belongs to the family of small-molecule inhibitors of the MDM2–p53 interaction (12). Nutlin-3 has been shown, alone or in combination with chemotherapeutic agents, to increase the degree of apoptosis in hematologic malignancies (13). Recent studies extended its therapeutic window for use in solid tumors (14, 15).

The aim of this study was to investigate the role of α5β1 integrin in glioma resistance to temozolomide chemotherapy using *in vitro* and *in vivo* models. We found that a high expression of α5 subunit inhibited the temozolomide-induced p53 pathway and that reactivation of p53 by nutlin-3a restores the sensitivity to temozolomide by decreasing the expression of the α5β1 integrin. Finally, we found that high α5 integrin gene expression is associated with a more aggressive phenotype in brain tumors and a decrease in survival of patients. Our results provide a clinical rationale for including α5β1 integrin–targeted therapy in a subpopulation of patients with glioma.

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Materials and Methods

Reagents

Temozolomide was a kind gift from Schering-Plough. Nutlin-3a, the active enantiomer of nutlin, was from Cayman. Temozolomide was prepared before use at 10 mmol/L in 50/50 ethanol/H_2O. Other drugs were prepared as stock solutions in ethanol at 10 mmol/L and were kept at −20°C until use.

Cell culture and transfection

The U87MG cells (p53-wt) was from American Type Culture Collection; the U373 cells (p53-mutated) from ECACC (Sigma) and not authenticated in the laboratory. The LN18 (p53-mutated) and LNZ308 [p53 knockout (KO)] cells were kindly provided by M. Hegi (University Hospital, Lausanne, Switzerland). Cells were cultured as described elsewhere (10). The identity of cell lines was regularly checked by morphologic criteria, and importantly p53 status was routinely checked by the yeast functional assay (16), Western blot quantification of p53 stability and phosphorylation, and by quantitative PCR (qPCR) quantification of p53 target genes after treatment with ellipticine. Cells were stably transfected to overexpress (by transfecting a pcDNA3.1 plasmid containing the human α5 integrin gene; provided by Dr. Ruoshlati, University of California, Santa Barbara, CA) or to repress [by transfecting a pSM2 plasmid coding for a short hairpin RNA (shRNA) targeting the α5 mRNA; Open Biosystems] the α5 integrin subunit by using jetPRIME (Polyplus transfection) according to the manufacturer's instructions. The vector for the p53-wt transfection was a kind gift from Dr. C. Blattner (Karlsruhe Institute of Technology, Karlsruhe, Germany). Cells were transfected with specific siRNA for human p53, the α5 integrin subunit, or nontargeting siRNA (Thermo Scientific Dharmacon) with jetPRIME (Polyplus transfection) according to the manufacturer's instructions.

Western blot

Western blotting was carried out as previously described (10). Antibodies used were against α5 integrin Ab1928 (Millipore) or H104 (Santa-Cruz), β1 integrin Ab1952, glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Millipore,), p53 (BD Biosciences), or $p53^{pser15}$ (Cell Signaling).

Flow cytometry

After detachment with EDTA, cells were incubated for 30 minutes at 4°C under agitation in the presence of primary antibodies: anti-α5 integrin antibody IIA1 (BD Biosciences) and anti-β1 integrin antibodies (TS2/16 from Santa Cruz; 9EG7 and mab13 from BD Biosciences). After washing, cells were incubated for 30 minutes with secondary antibody (Alexa488-conjugated goat anti-mouse or rat; Jackson ImmunoResearch). After washing, cells were analyzed using a FACSCalibur flow cytometer (Becton Dickinson), and the mean fluorescence intensity characterizing surface expression of integrins was measured using the CellQuest software.

Clonogenic assay

Clonogenic survival was determined as previously described (9).

Immunofluorescence

A total of 20,000 cells were seeded onto IBIDI μ-dishes coated with 10 μg/mL of poly-L-lysine. Cells were treated with nutlin-3a (10 μmol/L) or with solvent during 24 hours before fixation with 4% paraformaldehyde (10 minutes at room temperature) and then processed for α5 immunodetection (IIA1 antibody; 1:300). Confocal images were taken with a confocal microscope (BioRad 1024) equipped with a water immersion ×60 objective. Images were collected using the Laser-Sharp 2000 software.

Human biopsies

This study was conducted on 115 adult brain biopsies, 95 brain tumors (22 grade II, 38 grade III, and 35 grade IV) and 20 nontumoral brain tissues collected retrospectively from archival material stored at the Centre de Ressources Biologiques et Tumorothèque (Hopitaux Universitaires de Strasbourg, Strasbourg, France). The patient characteristics have been described elsewhere (17). Each sample was histologically analyzed by a pathologist to specify the tumor grade and the percentage of tumor cells. Only samples with at least 50% of tumoral cells (>50% of samples were >70% tumoral cells) have been included in the study. Control tissues were obtained from epileptic surgery. The study was conducted in accordance with the Declaration of Helsinki. Real-time qPCR was carried out as described previously (17). The threshold cycle (C_t) values for each gene were normalized to expression level of *cyclophilin* used as the housekeeping gene. Values were normalized relatively to the value obtained for one nontumoral control brain tissue, which was included in each qPCR run. Immunologic analysis of α5 protein expression was conducted as shown previously (17).

Human brain tumor data sets

Glioma gene expression data sets from 2 other cohorts were downloaded from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo; accession numbers GSE4271 and GSE4412). Microarray raw data were processed using R (version 2.10.1; http://cran.r-project.org/), implemented with the BioConductor package (http://www.bioconductor.org). Estimates of the survival curves were computed using the Kaplan–Meier method. Univariate survival comparisons between the patients, according to low or high α5 integrin expression levels, were conducted using a log-rank test.

Human brain tumor xenografts

TCG4, TCG9, and TCG17 glioma xenograft models were obtained as previously described (18). Subcutaneous tumor growth was followed by measuring, 3 times per week, 2 perpendicular diameters with a caliper. Treatments began when tumors reached a volume of approximately 250 ± 50 mm^3. Temozolomide was administered orally at the dose of 50 mg/kg/d for 5 days. Mice were sacrificed when the tumor volumes reached 4 times their initial volume (V_0). For each mouse, the time between the treatment onset and the animal sacrifice was defined as the "survival time." *TP53* status of each xenograft was determined by the yeast functional assay (16).

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Statistical analysis

Data are represented as the mean ± SEM, and n is the number of independent experiments. Statistical analyses were conducted using the Student t test or the Mann–Whitney test with the GraphPad Prism program. $P < 0.05$ was considered significant.

Results

α5β1 integrin impedes temozolomide-induced p53-wt activity

We compared the effect of temozolomide in U87MG cells depleted in ($shRNA_{\alpha5}$) or overexpressing ($pcDNA_{\alpha5}$) α5 integrin versus control cells ($shRNA_{ns}$ and $pcDNA_{ctrl}$, respectively). Because p53 is largely involved in chemotherapeutic drug effects and we showed previously that α5β1 integrin antagonists modulate the p53 pathway (10), we focused on temozolomide-induced p53 activation. Temozolomide caused an increase in p53 protein in all cell lines but not significantly in $pcDNA_{\alpha5}$ cells (when normalized to GAPDH: Fig. 1A). Interestingly, a significant increase in p53 protein was already observed in untreated $shRNA_{\alpha5}$ cells versus $shRNA_{ctrl}$ cells (Fig. 1A). After temozolomide treatment, an increase in $p53^{pser15}$ was detectable in $pcDNA_{ctrl}$ and $shRNA_{ns}$ cells, which was significantly more pronounced in $shRNA_{\alpha5}$ (Fig. 1A). In contrast, in $pcDNA_{\alpha5}$ cells, significantly less $p53^{pser15}$ was measured after temozolomide treatment. Transcriptional activity of p53 was higher in $shRNA_{\alpha5}$ cells and lower in $pcDNA_{\alpha5}$ cells than in temozolomide-treated control cells (Fig. 1B). Taken together, these results indicate that α5β1 integrin modulates p53 activity and that high expression of this integrin inhibits temozolomide-induced p53 stimulation. Modulation of p53 activity was related to cell survival, as $pcDNA_{\alpha5}$ cells are significantly more resistant at high temozolomide concentration whereas $shRNA_{\alpha5}$ cells appear more sensitive than their control counterparts (Fig. 1C). α5 integrin overexpression did not modulate p53, nor clonogenic survival in U373 and LN18 cells expressing a p53-mutant (Supplementary Fig. S1). In addition, repression of α5 integrin in p53-deficient LNZ308 cells did not sensitize cells to temozolomide (Supplementary Fig. S1). From these data, we concluded that α5β1 integrin–induced temozolomide resistance requires a functional p53.

As a first approach to confirm the role of the α5β1 integrin in temozolomide chemoresistance *in vivo*, we used subcutaneous xenografted human brain tumors in nude mice. We selected 3 xenografts that exhibited a wild-type p53 and different levels of the α5 subunit. Kaplan–Meier analysis of the mouse survival suggests a relationship between α5 integrin level and resistance to temozolomide (Fig. 1D), providing some evidence for a role of the α5 integrin in the chemoresistance of p53-wt–expressing tumors *in vivo*.

Activation of p53 by nutlin-3a overrides the α5 integrin effects

We next investigated whether high α5 also impacts on p53 activation by a non-genotoxic p53 activator in glioma cells. U87MG cells were treated with nutlin-3a. In contrast to the effects of temozolomide, nutlin-3a stabilized p53, markedly increased the $p53^{pser15}$ and the transactivation of p53 target genes in $pcDNA_{ctrl}$ and in $pcDNA_{\alpha5}$ cells (Fig. 2A and B). Addition of temozolomide to nutlin-3a does not further increase these effects (Fig. 2A). In clonogenic assays, the α5β1 integrin did not efficiently protect the cells from death when p53 was activated by 10 μmol/L nutlin-3a (Fig. 2C). Survival of LNZ308 cells (p53 KO) or U373 cells (p53-mutant) was less affected after treatment with nutlin-3a (Fig. 2C).

Activation of p53 by nutlin-3a markedly decreases the α5 expression level in glioma cells

U87MG cells treated with nutlin-3a rounded up and detached from the wells. This effect was lost when p53 expression was inhibited with specific siRNA (Fig. 3A, left). In contrast, LNZ308 cells did not exhibit any morphologic alterations after a 10 μmol/L nutlin-3a treatment unless p53 was reexpressed in the cells (Fig. 3A, right). Interestingly, cell treatment with nutlin-3a decreased the expression of the α5 integrin at the protein level in U87MG-$pcDNA_{ctrl}$ and U87MG-$pcDNA_{\alpha5}$ cells, an effect not observed after treatment with temozolomide (Fig. 3B). The decrease in α5 protein expression after nutlin-3a treatment was confirmed by specific immunostaining of the α5 subunit in U87MG-$pcDNA_{ctrl}$ and U87MG-$pcDNA_{\alpha5}$ cells and by flow cytometric analysis of the α5 subunit at the cell membrane (Fig. 3B). A significant decrease in the α5 mRNA level was measured in U87MG $pcDNA_{ctrl}$ but not in $pcDNA_{\alpha5}$ cells, suggesting that nutlin-3a affected the α5 subunit at translational and posttranslational levels (Fig. 3C). Nutlin-3a also decreased β1 at the protein and mRNA level in the U87MG cells, suggesting that both subunits of the α5β1 integrin are processed similarly after nutlin-3a treatment (Supplementary Fig. S2). However, no effect on the β1 subunit expressed at the cell membrane could be detected after nutlin-3a treatment (Supplementary Fig. S2 and Supplementary Table S1).

Nutlin-3a did not affect the endogenous α5 protein in p53-knockout LNZ308 cells unless p53 was reexpressed (Fig. 3D). Nutlin-3a had no effect on α5 expression in U373 cells (Supplementary Fig. S3). Altogether, these data suggest that nutlin-3a requires a functional p53 to decrease α5 expression, which in turn make the cells susceptible to the nutlin-3a–induced cell death.

High α5β1 integrin expression is associated with worse clinical outcome in high-grade glioma

To our knowledge, no studies have associated α5β1 integrin with clinical outcome in patients with glioma. To investigate first whether integrin expression is associated with the grade of brain tumors, gene expression of the α5 and β1 subunits were examined by qPCR in 95 human brain tumors of different grades and compared with 20 nontumor brain samples. The data revealed that α5 subunit gene expression was increased with increasing tumor grade, although the β1 subunit was equally overexpressed in the 3 tumoral grades compared with control tissue (Fig. 4A). Data were confirmed at the protein level (Fig. 4B). Because the α5 subunit only dimerizes with β1,

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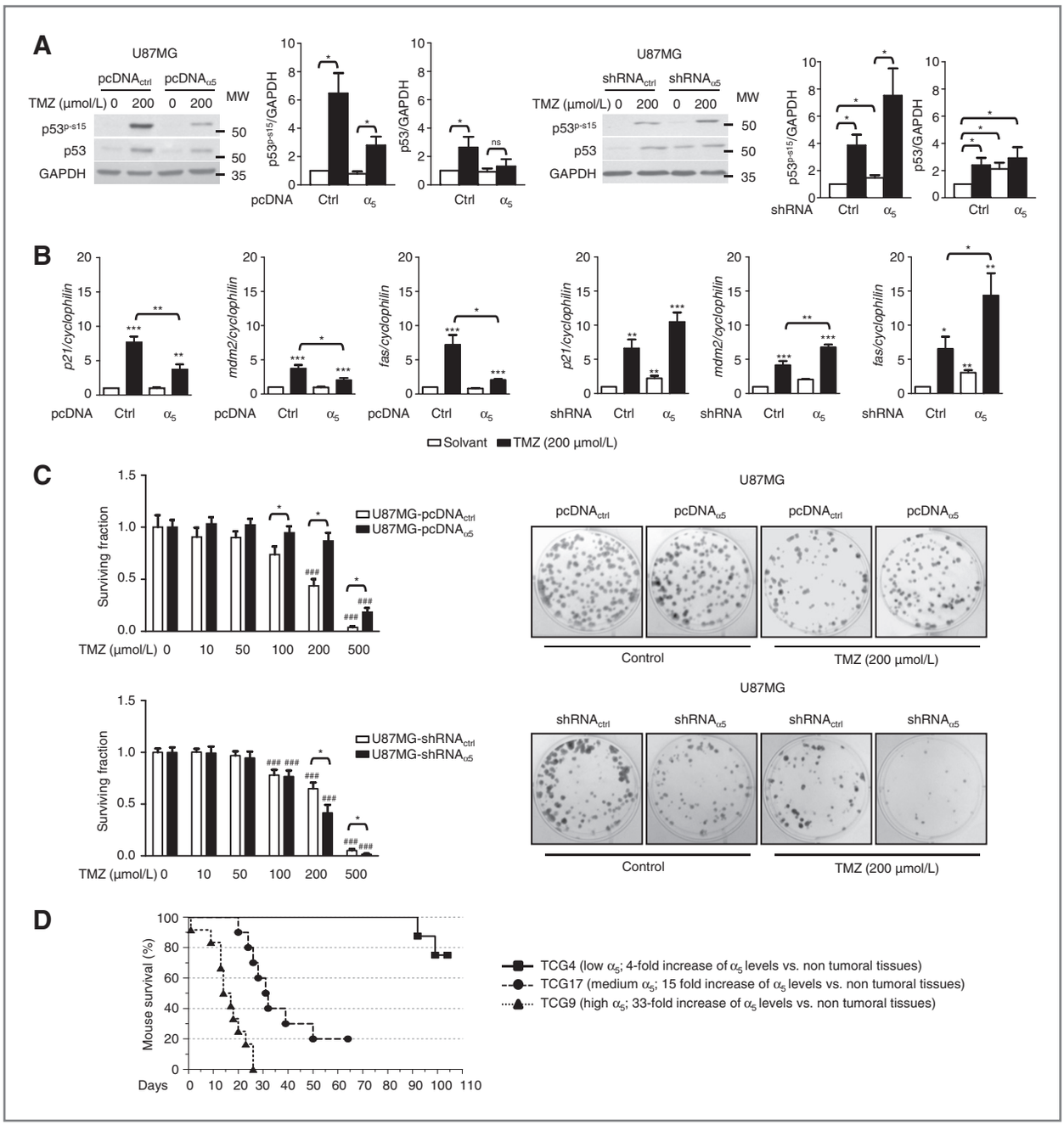

Figure 1. Elevated α5 integrin expression impairs temozolomide (TMZ)-induced p53-wt signalling and triggers TMZ resistance. A, stability and p53 phosphorylation on Ser15 are affected by the α5 integrin expression level. Western blot analysis for p53 and p53^{pser15} from total cell lysates with and without TMZ (200 μmol/L) treatment during 24 hours in control (pcDNA$_{ctrl}$) and α5 integrin–overexpressing (pcDNA$_{\alpha5}$) U87MG cells (top) or control (shRNA$_{ns}$) and α5 integrin–downregulated (shRNA$_{\alpha5}$) U87MG cells (bottom). Histograms represent the mean ± SEM of 6 to 8 independent experiments. B, qPCR quantification of p53 target genes. mRNA of target genes are differentially affected by upmodulated (top) or downmodulated (bottom) α5 integrin after TMZ treatment in U87MG cells. C, TMZ dose response of clonogenic survival in U87MG cells overexpressing α5 integrin (U87-pcDNA$_{\alpha5}$) compared with control cells (U87-pcDNA$_{ctrl}$). pcDNA$_{\alpha5}$ cells are 2 times more resistant than control cells at 200 μmol/L TMZ (top) or U87MG cells underexpressing α5 integrin (U87-shRNA$_{\alpha5}$) compared with control cells (U87-shRNAns). shRNA$_{\alpha5}$ are 1.5 times more sensitive than control cells at 200 μmol/L TMZ (bottom). Representative images of colonies obtained with and without 200 μmol/L TMZ are shown. Statistical analysis: ##, $P < 0.01$; ###, $P < 0.001$ for treated cells versus nontreated cells; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ for genetically manipulated cells versus corresponding control cells. D, TMZ antitumor effect on human malignant glioma xenografts in nude mice. Three glioma xenografts expressing p53-wt were analyzed for α5 mRNA expression (TCG9, TCG17, and TCG4 with 33-, 15-, and 4.5-fold more α5 mRNA, respectively, compared with human nontumor brain tissue) and used to evaluate the tumor response to TMZ (orally daily 50 mg/kg × 5 days). Results are expressed as Kaplan–Meier plots, considering the percentage of tumors that reached four V_0 as the survival end point. Ctrl, control; ns, not significant.

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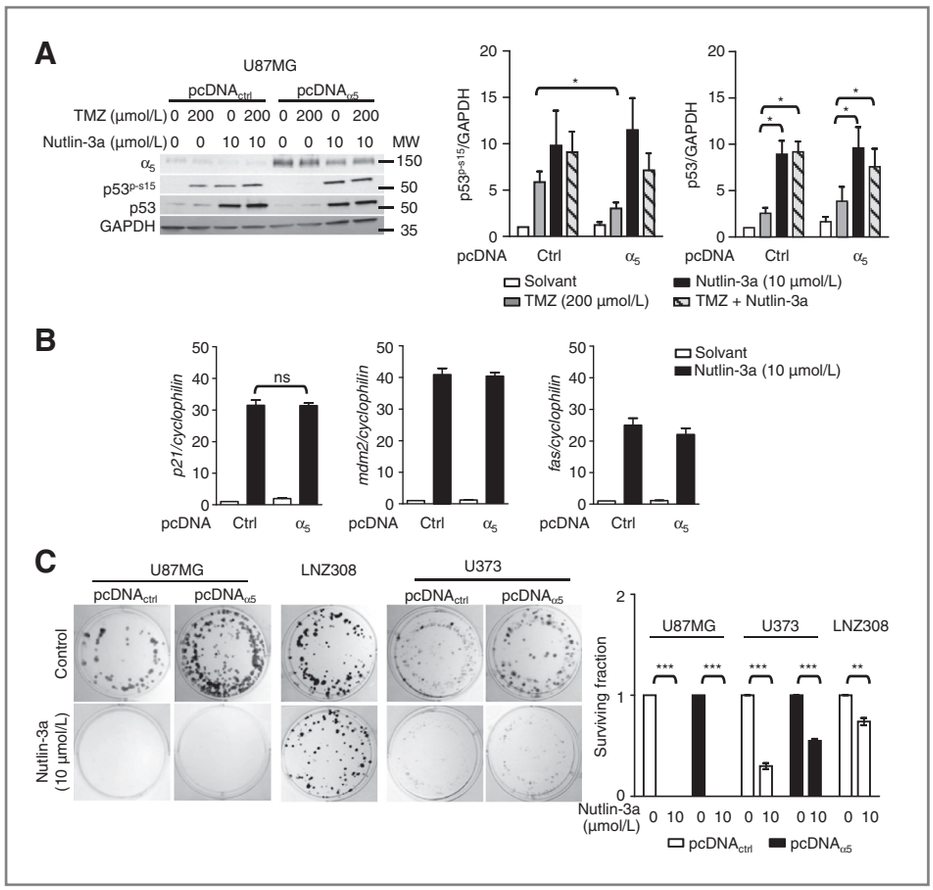

Figure 2. Activation of p53 by nutlin-3a overrides the inhibitory effect of the α5 integrin. A, Western blot analysis of the α5 integrin, p53, and p53^{pser15} in pcDNA$_{ctrl}$ and pcDNA$_{α5}$ -transfected U87MG cells. U87MG cells were treated with nutlin-3a (10 μmol/L), temozolomide (TMZ; 200 μmol/L), or both for 24 hours. The histograms display the mean ± SEM of 5 independent experiments. GAPDH was used as the loading control. B, qPCR analysis of p53 target genes. The histograms represent the fold increase of mRNA in pcDNA$_{ctrl}$ and pcDNA$_{α5}$ -transfected U87MG cells before and after nutlin-3a (10 μmol/L) treatment over 24 hours. ns, nonsignificant. C, clonogenic survival of pcDNA$_{ctrl}$ -U87MG and pcDNA$_{α5}$-U87MG cells (left), LNZ308 cells (middle), and pcDNA$_{ctrl}$-U373 and pcDNA$_{α5}$ -U373 cells (right) after nutlin-3a treatment (10 μmol/L). Histograms represent the mean ± SEM. *, $P < 0.05$ of 3 independent experiments. **, $P < 0.01$; ***, $P < 0.001$ in nutlin-3a–treated versus nontreated cells.

the data point toward a particular role for α5β1 integrin in glioma.

We analyzed the clinical data of grade III and grade IV patients. Log-rank analysis of the Kaplan–Meier survival curves showed a significant survival advantage for patients with low α5-expressing glioma compared with high α5-expressing glioma. These results were validated in 2 independent public data sets (2, 19). Considering all 3 cohorts together, the group of high α5-expressing tumors included 39% of grade III and 81% of grade IV tumors (Fig. 4C; Supplementary Table S2). Finally, we evaluated the relationship between α5 integrin level and the status of p53 in 56 human biopsies (grade III and IV) and in 17 human tumor xenografts. A clear tendency toward a higher level of α5 in p53-wt versus p53-mutant tumors was found in biopsies and in xenografts (Fig. 4D; Supplementary Table S3).

Discussion

The data summarized here document the impact of the α5β1 integrin on the high-grade glioma resistance to temozolomide therapy. When the α5 integrin subunit is overexpressed, the p53-mediated responses to genotoxic damage are compromised. When the α5 integrin level is low or suppressed, p53 is stabilized and fully functional. An inverse relationship between the α5 integrin level and p53 has been revealed through the use of the p53 activator, nutlin-3a. These results may have clinical relevance in light of the clear advantage reported here for prolonged survival of patients with high-grade glioma with low α5 integrin subunit expression.

In agreement with our data, it was reported that the α5β1 integrin is overexpressed at the protein level in a significant proportion of human glioblastoma biopsies (20). Here, we show for the first time that in glioma, the α5 mRNA level is negatively correlated to survival in 3 different cohorts of patients, which adds brain tumors to the growing list of cancers in which the α5β1 integrin should be considered as a therapeutic target. The role of p53 in temozolomide resistance is far from being understood. Although several groups reported that p53 status is not predictive of response to chemotherapy with alkylating agents (18, 21), more recent works suggest that the absence of a functional p53 increases temozolomide sensitivity in glioma cell lines (22) and in intracranial glioblastoma xenografts (23). A trend toward an increased temozolomide sensitivity in patients with p53 mutations was also suggested (24). We propose that overexpression of the α5β1 integrin in GBM represents an alternative mechanism, aside from p53 deletion/mutation, to inactivate the tumor-suppressive function of the p53 pathway. We are currently investigating the molecular mechanisms involved in the integrin–p53 cross-talk by addressing the role of α5β1 integrin in transcriptional and nontranscriptional effects of p53. Activation of the p53 pathway by nutlin-3a led to downregulation of

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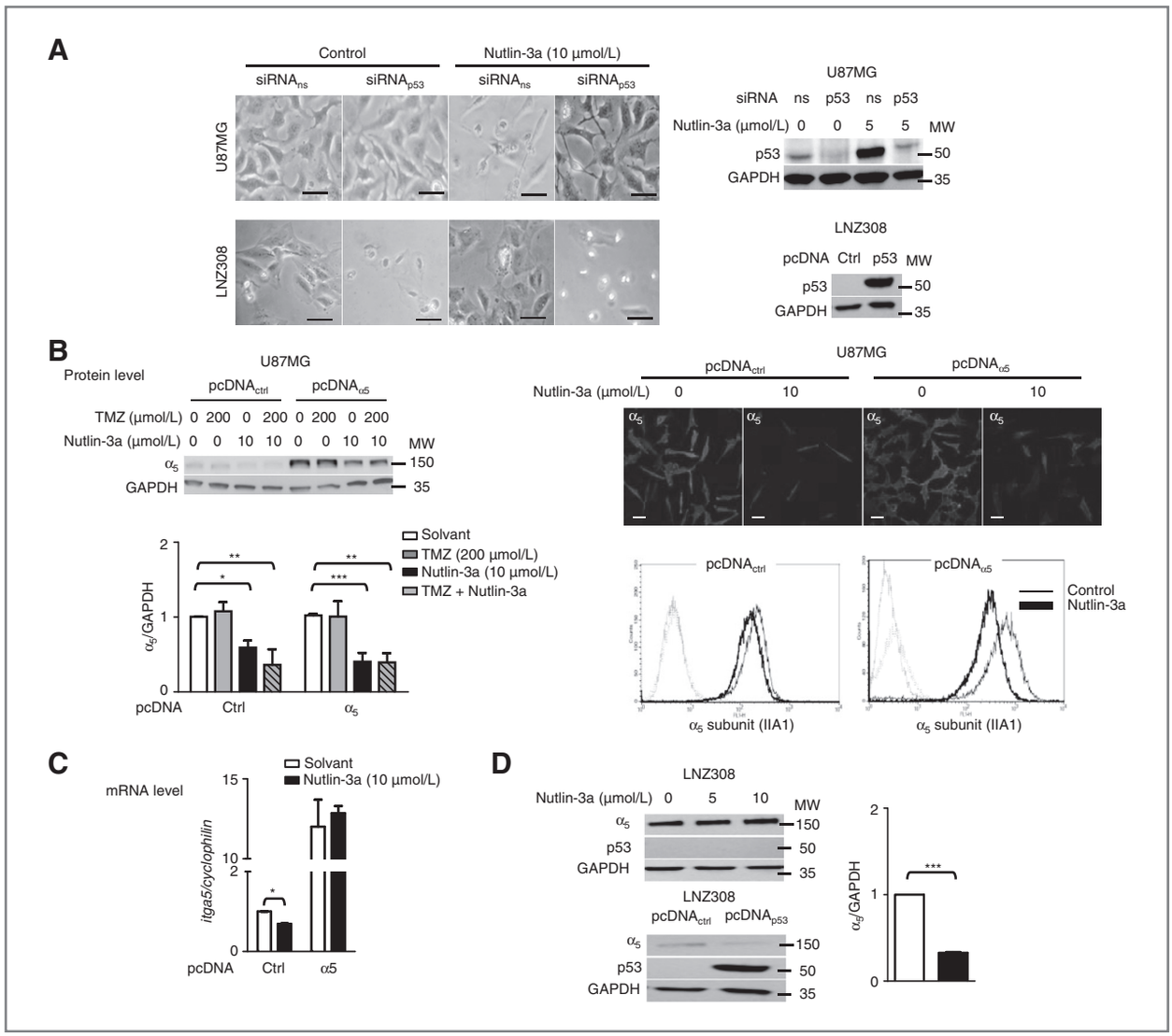

Figure 3. Activation of p53 by nutlin-3a affects the α5β1 integrin expression in U87MG cells. A, U87MG and LNZ308 cell morphology after 24 hours of nutlin-3a treatment. U87MG cells were transfected either with control $siRNA_{ns}$ or with $siRNA_{p53}$ and treated with nutlin-3a (5 μmol/L) for 24 hours. Silencing of the p53 protein was verified by immunoblotting. LNZ308 cells were transfected with $pcDNA_{ctrl}$ or $pcDNA_{p53}$ and treated with nutlin-3a (10 μmol/L). Expression of p53 was verified by immunoblotting. Scale bars, 50 μm. B, top, Western blot analysis of the α5 integrin protein expression in $pcDNA_{ctrl}$- and $pcDNA_{α5}$-transfected U87MG cells. Cells were treated with nutlin-3a (10 μmol/L), TMZ (200 μmol/L), or both drugs for 24 hours. Histograms show the fold increase in the protein expression normalized to GAPDH levels (mean ± SEM of 3–4 independent experiments). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ for treated cells versus nontreated cells. Middle, representative fluorescence images with specific anti-α5 integrin antibodies of untreated and nutlin-3a-treated $pcDNA_{ctrl}$- and $pcDNA_{α5}$-transfected U87MG cells. Scale bars, 20 μm. Bottom, flow cytometric analysis of the α5 (IIA1 antibody) integrin subunit at the cell membrane of $pcDNA_{ctrl}$- and $pcDNA_{α5}$-transfected U87MG cells before and after nutlin-3a (10 μmol/L) treatment for 24 hours. C, histograms represent the fold increase of the α5 mRNA in $pcDNA_{ctrl}$- and $pcDNA_{α5}$-transfected U87MG cells after nutlin-3a (10 μmol/L) treatment. *, $P < 0.05$ for treated cells versus nontreated cells. D, left, Western blot analysis of α5 and p53 protein expression in p53-null LNZ308 cells treated with nutlin-3a (5 and 10 μmol/L) for 24 hours. A representative blot of 3 is shown. Right, LNZ308 cells were transfected with p53-wt, and α5 integrin expression was detected by Western blot analysis. Histogram represents the mean ± SEM of 3 independent experiments. GAPDH was used as the loading control. Ctrl, control; ns, not significant.

the α5 integrin subunit in glioma cells. On the basis of our data, there seems to be a cross-antagonistic interaction between the α5 integrin and p53 that was only revealed by nutlin-3a, which may explain why this drug overcomes the prosurvival activity of the integrin. Our results are similar to recent data showing that nutlin-3a downregulates the oncogene DEK or overcomes the antiapoptotic Bcl2 overexpression, thus leading to cell apoptosis (25, 26).

In summary, we have shown for the first time that α5β1 integrin plays a critical role in resistance to temozolomide therapy by interfering with the p53 pathway in high-grade glioma. In addition, we have shown that activation of p53 by

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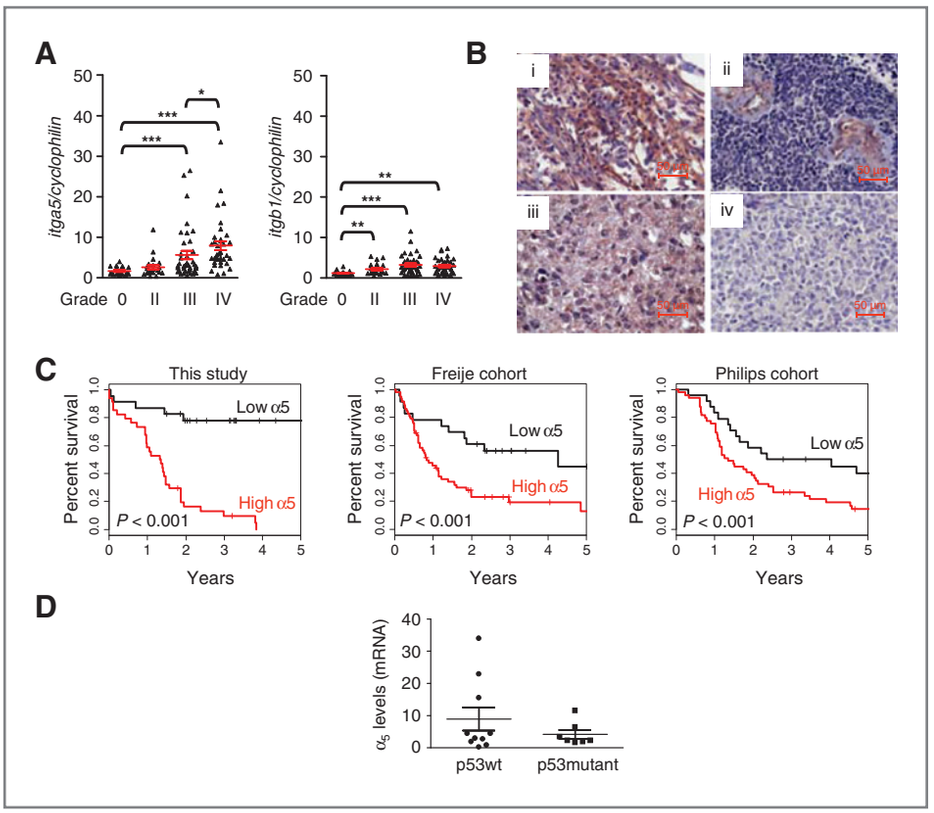

Figure 4. Elevated α5-integrin gene expression is associated with high-grade glioma and predicts decreased survival rates. A, gene expression levels of α5 and β1 integrin subunits were quantified with specific primers by qPCR in 20 nontumor brain tissues (G0), 22 grade 2 (GII), 38 grade 3 (GIII), and 35 glioblastoma (GIV) samples. Only the α5 integrin subunit level is associated with the tumor grade. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ as compared with nontumoral brain tissue (Mann–Whitney test). B, immunohistochemical analysis of α5 protein in tumoral cells of human high-grade glioma. Representative slides of α5 high intensity staining in GBM (i), α5-negative staining in GBM (ii), α5 high intensity staining in grade III tumor (iii), and α5-negative staining in grade III tumors (iv). Scale bars, 50μm. C, elevated α5 integrin gene expression is associated with significantly decreased long-term survival in patients with high-grade glioma. Kaplan–Meier survival analysis of 3 patient cohorts is shown. Left, cohort from this study; middle, data from Freije and colleagues (2); right, data from Phillips and colleagues (19). D, the α5 mRNA expression level and p53 status in human brain tumor xenografts in nude mice. Ten xenografts expressed a wild-type p53 and 7 xenografts had a mutant p53, as determined by the FASAY assay. The α5 mRNA levels were determined in at least 3 different grafts of the same tumor, and the mean levels were plotted according to p53 status. The mean values ± SEM of the α5 mRNA level in p53-wt and mutant p53 tumors were 8.9 ± 3.6 and 4.1 ± 1.3, respectively. Although not significant, this difference shows a trend toward an increased level of α5 in p53-wt tumors.

nutlin-3a represses the α5β1 integrin, and we propose that such downregulation is an important mediator of nutlin-3a cytotoxic activity. The relevance of our results is emphasized by the finding that α5 integrin gene overexpression is associated with decreased survival in patients with high-grade glioma. Our data provide the rationale for a preclinical evaluation of p53 activators and/or α5β1 integrin antagonists in a subset of high-grade glioma that expresses a functional p53 and high levels of α5β1 integrin.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: H. Janouskova, S. Pinel, P. Chastagner, M. Dontenwill
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Maglott, D.Y. Leger, C. Bossert, F. Noulet, E. Guerin, S. Pinel, F. Plenat, N. Entz-Werle, J. Lehmann-Che
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Janouskova, A. Maglott, F. Noulet, P. Chastagner, F. Plenat, J. Godet, M. Dontenwill
Writing, review, and/or revision of the manuscript: H. Janouskova, D. Guenot, P. Chastagner, F. Plenat, J. Lehmann-Che, J. Godet, S. Martin, M. Dontenwill
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Guenot, N. Entz-Werle, S. Martin
Study supervision: D. Guenot, P. Chastagner, M. Dontenwill

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