EXPERIMENTAL CELL RESEARCH 318 (2012) 925–935

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/yexcr

Research Article

Quercetin abrogates IL-6/STAT3 signaling and inhibits glioblastoma cell line growth and migration

*Jonathan Michaud-Levesque, Nathalie Bousquet-Gagnon, Richard Béliveau**

Laboratoire de Médecine Moléculaire, Université du Québec à Montréal, C.P. 8888, Succ. Centre-ville, Montréal, Québec, Canada H3C 3P8

ARTICLE INFORMATION

Article Chronology:
Received 3 November 2011
Revised version received
13 February 2012
Accepted 20 February 2012
Available online 26 February 2012

Keywords:
Quercetin
Interleukin-6
STAT3
Cyclin D1
MMP-2
Cell growth and migration
Glioblastoma
Cancer

ABSTRACT

Evidence has suggested that STAT3 functions as an oncogene in gliomagenesis. As a consequence, changes in the inflammatory microenvironment are thought to promote tumor development. Regardless of its origin, cancer-related inflammation has many tumor-promoting effects, such as the promotion of cell cycle progression, cell proliferation, cell migration and cell survival. Given that IL-6, a major cancer-related inflammatory cytokine, regulates STAT3 activation and is upregulated in glioblastoma, we sought to investigate the inhibitory effects of the chemopreventive flavonoid quercetin on glioblastoma cell proliferation and migration triggered by IL-6, and to determine the underlying mechanisms of action. In this study, we show that quercetin is a potent inhibitor of the IL-6-induced STAT3 signaling pathway in T98G and U87 glioblastoma cells. Exposure to quercetin resulted in the reduction of GP130, JAK1 and STAT3 activation by IL-6, as well as a marked decrease of the proliferative and migratory properties of glioblastoma cells induced by IL-6. Interestingly, quercetin also modulated the expression of two target genes regulated by STAT3, i.e. cyclin D1 and matrix metalloproteinase-2 (MMP-2). Moreover, quercetin reduced the recruitment of STAT3 at the *cyclin D1* promoter and inhibited Rb phosphorylation in the presence of IL-6. Overall, these results provide new insight into the role of quercetin as a blocker of the STAT3 activation pathway stimulated by IL-6, with a potential role in the prevention and treatment of glioblastoma.

Introduction

Several studies have associated abundant intake of foods of plant origin with a substantial reduction in risk of developing various cancers [1]. Flavonoids are polyphenolic substances, widely distributed in almost every food plant, that possess antiviral, antimicrobial, anti-inflammatory, anti-allergic, anti-thrombotic, antimutagenic, antineoplasic, and cytoprotective effects on different cell types, both in animal and human models [2]. Epidemiologic studies have suggested that high consumption of flavonoids may be associated with decreased risk of several types of cancer [3]. Many of the reports investigating flavonoids highlight quercetin [4]. Quercetin is found in a variety of plant-based foods such as red onions, apples, tea (*Camelia sinensis*), broccoli, capers, lovage, parsley, red grapes and a number of berries [5]. The potential chemopreventive effects of quercetin have been attributed to various mechanisms including its anti-oxidative activity as well as its capacity to inhibit enzymes that activate carcinogens, to modify signal transduction pathways, and to interact with and regulate cell receptors and other proteins [6]. Although the pharmacological

* *Corresponding author.* Fax: +1 5149870246.
E-mail address: oncomol@nobel.si.uqam.ca (R. Béliveau).

0014-4827/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2012.02.017

interest of quercetin and its possible nutritional benefit, the molecular mechanisms underlying its chemopreventive activities still remain poorly understood.

Over the past decade, the incidence of primary brain tumors has rapidly increased, and glioblastoma remains the most frequent and malignant histological type of all brain tumors [7,8]. Despite advances in surgical and medical therapy, glioblastoma have retained their poor prognosis. Therefore, it is important to comprehend their underlying mechanisms in order to identify new therapeutic strategies against glioblastoma.

Interleukin-6 (IL-6), also known as interferon-beta 2, is a pleiotropic cytokine primarily involved in the regulation of immuno- and inflammatory responses. IL-6 is widely expressed by a large variety of malignant tumors, including prostate, breast, lung cancer and glioblastoma [9]. IL-6 is a key cytokine that orchestrates the inflammatory microenvironment surrounding the tumor. Many studies have shown that IL-6 contributes to malignant progression by the regulation of a variety of biological processes, such as proliferation, apoptosis and survival as well as invasion [10]. In patients with glioblastoma, the expression level of IL-6 has been suggested as being related to the prognosis of the patients [11], and IL-6 was shown to promote the proliferation of glioma cell lines [12]. However, whether IL-6 could contribute to the proliferative and migratory abilities of glioblastoma must be better understood before more effective treatment strategies for these tumors can be found.

Several IL-6-related signaling pathways have been identified and associated with increased proliferation, migration and invasion of various tumor cells. Briefly, IL-6 binds to an IL-6-specific binding receptor (IL-6Rα) and triggers the dimerization of the signal transducer receptor (GP130, also called CD130), leading to its phosphorylation and the subsequent activation of Janus tyrosine kinase (JAK) [13]. These events lead to the activation of several signal-transduction pathways, such as the signal transducer and activator of transcription (STAT), Ras-MAPK and PI-3 kinase signaling pathways [13]. Among them, the STAT3 signaling pathway is one of the most studied cytokine signaling systems [14,15]. STAT3, a key cytoplasmic transcription factor involved in inflammation becomes activated in response to a variety of cytokines, chemokines and growth factors. STAT3 is constitutively active in various human malignancies, including breast and pancreatic cancer as well as glioblastoma [16]. STAT3 activation requires phosphorylation of the residue Tyr705, leading to protein dimerization and translocation from the cytoplasm to the nucleus [17]. Activation of STAT3 is linked to clinically more aggressive phenotypes of glioblastomas [18]. Constitutive STAT3 signaling has been shown to contribute to cancer progression by the regulation of various target genes involved in the regulation of critical cellular processes regulating gliomagenesis, such as promoting cell cycle progression, angiogenesis, cell migration/invasion and immune evasion, as well as preventing apoptosis [16]. Moreover, cyclin D1 is reported to be transcriptionally regulated by STAT3 is frequently deregulated in various cancers [15,19–21] and is directly involved in the regulation of Rb phosphorylation [22]. Retinoblastoma (Rb) phosphorylation plays a crucial role in the progression of G_1 phase and the transition of G_1 to S phase [23], and hypo-phosphorylation of the Rb protein has been linked to G_0/G_1 arrest [24]. Also, it has been shown that STAT3 directly regulates MMP-2 expression as well as tumor cell migration, invasion and metastasis [15,25–27]. Therefore STAT3 can be considered as an oncogenic transcription factor due to its ability to promote malignancy [10,28,29]. Since STAT3 plays a central role in glioblastoma signal transduction, it has promising potential as a therapeutic target for glioma therapy.

In this study, we show that quercetin acts as a potent inhibitor of the JAK/STAT3 signaling pathway stimulated by IL-6. The inhibition of IL-6-induced STAT3 phosphorylation by quercetin strongly reduces glioblastoma cell proliferation and migration, mainly through the downregulation of two STAT3 target genes, i.e. Cyclin D1 and MMP-2. Moreover, STAT3 associates with the *cyclin D1* promoter in the presence of IL-6, and quercetin antagonized this recruitment. Altogether, these results highlight quercetin as an efficient, dietary-derived inhibitor of the IL-6/STAT3 signaling and suggest that this inhibitory effect may contribute to the chemopreventive properties of quercetin.

Materials and methods

Materials

Human recombinant IL-6 was from R&D Systems (Minneapolis, MN). Specific antibodies against STAT3 (#4904), phospho-STAT3 (Y705, #9145), GP130 (#3732), JAK1 (#3344), phospho-JAK1 (Y1022/1023, #3331), phospho-JAK2 (Y1007/1008, #3776), cyclin D1 (#2926), CDK4 (#2906), Rb (#9309), and phospho-Rb (S780, #9307 and S795, #9301) were from Cell Signaling Technology (Pickering, ON). Antibodies against JAK2 (sc-294) and phospho-GP130 (S782, sc-22346) were from Santa Cruz Biotechnology (Santa-Cruz, CA) whereas antibodies against IL-6Rα (AF-227-NA), α-tubulin (T8660) and GAPDH (RGM2) were from R&D Systems (Minneapolis, MN), Sigma-Aldrich (Oakville, ON) and Advanced ImmunoChemical (Long Beach, CA), respectively. Non-specific IgG antibodies and other biochemical reagents were from Sigma-Aldrich.

Cell culture

U87 and T98G human glioblastoma cells were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) whereas U251 human glioblastoma cells were obtained from the NCI-Frederick Cancer DCTD Tumor/Cell Line Repository (Bethesda, MD). Cells were cultured under a 5% CO_2/95% air atmosphere. U87 cells were cultured in Eagle's Minimum Essential Medium (EMEM) (Wisent; St-Bruno, QC), containing 1 mM pyruvate, 100 U/mL penicillin-streptomycin and 10% calf bovine serum (Hyclone; Logan, UT, USA). T98G cells were cultured in EMEM (Wisent) containing 100 U/mL penicillin-streptomycin and 10% calf bovine serum. U251 cells were cultured in RPMI-1640 (Wisent) containing 100 U/mL penicillin-streptomycin and 10% calf bovine serum. To normalize each different experimental condition and to reduce the effect of cell density on STAT3, all cellular assays were conducted at 80% confluence throughout the study.

Western blot analysis

Cells were collected by trypsinization, washed with PBS and lysed in lysis buffer (150 mM NaCl, 10 mM Tris–HCl, pH 7.4, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ethylene glycol-bis-(2-aminoethyl)-N,N,N′,N′-tetraacetic acid (EGTA), 0.5% Nonidet P-40, 1% Triton X-100, 1 mM Na_3VO_4, 1 mM NaF and containing a protease

inhibitor cocktail set (#539131; Calbiochem, Darmstadt, Germany)). Cell lysates (20 μg to 40 μg) were solubilized in Laemmli sample buffer (125 mM Tris–HCl (pH 6.8), 20% glycerol, 4% SDS, 10% β-mercaptoethanol, and 0.00125% bromophenol blue), boiled for 4 min, and subjected to sodium dodecyl-sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins were then electro-transferred to polyvinylidene difluoride (PVDF) membranes. Following transfer, immunodetection analysis was performed and the immunoreactive bands were visualized by enhanced chemiluminescence.

Cell proliferation assay

The effects of quercetin on IL-6-induced U87 and T98G cell proliferation was measured by [^{3}H]-thymidine incorporation assay. Briefly, 5×10^3 cells were seeded onto 96-well plates. After 24 h, they were serum-starved for 20 h, and then treated with various doses of quercetin in the presence of IL-6 (100 ng/mL) for an additional 48 h. The experiments were initiated by adding 0.5 μCi/well of [^{3}H]-thymidine (Perkin Elmer; Boston, MA) and cells were incubated for 4 h at 37 °C in 5% CO_2/95% air. Afterwards, cells were washed two times with PBS (37 °C), fixed 5–10 min in EtOH/acetic acid (3:1) and air-dried. The amount of [^{3}H]-thymidine incorporated into the cells was measured with Microscint-0 (Perkin Elmer; Waltham, MA) using a liquid scintillation counter.

Cell cycle and apoptosis FACS analysis

All flow cytometry measurements were performed with the FACS-Calibur™ flow cytometer (BD Biosciences; Mississauga, ON). The data were analyzed with BD CellQuest Pro software. For the cell cycle analysis, T98G and U87 cells were treated for 24 h in serum-free media with or without IL-6 (100 ng/mL) and quercetin (25 μM). For all cell cycle experiments, both cells growing on the surface of the dishes and in the culture medium were harvested, fixed with 75% ethanol, and stored at −20 °C for less than 1 week before staining and analysis. For the cell cycle analysis, cells were then resuspended in PBS containing PI at 10 μg/mL and RNase A at 0.2 mg/mL. After 45 min of incubation at room temperature, the cells were subjected to flow cytometry analysis. For the cell apoptosis analysis, T98G and U87 cells were treated for 24 h in serum-free media with or without IL-6 (100 ng/mL) and various concentrations of quercetin. Apoptosis analysis was performed using the BD Pharmingen FITC Annexin V Apoptosis Detection Kit (cat #556547). For all FACS experiments, more than 10,000 cells per condition were analyzed by flow cytometry.

Total RNA isolation and reverse transcription polymerase chain reaction (RT-PCR)

U87 and T98G cells were plated onto 55-cm Petri dishes and treated for 24 h in serum-free media with or without IL-6 (100 ng/mL) and quercetin (25 μM). Then total RNA was extracted from cells using TRIzol reagent (Invitrogen; Burlington, ON) following standard procedures. RT-PCR reactions were performed using SuperScript One-Step RT-PCR (Invitrogen). The primers used were as follows: cyclin D1, forward, 5′-AGA CCT GCG CGC CCT CGG TG-3′, and reverse 5′-GTA GTA GGA CAG GAA GTT GTT C-3′ (expected product 574 bp); GAPDH, forward 5′-CCA TCA CCA TCT TCC AGG AG-3′, and reverse 5′-CCT GCT TCA CCA CCT TCT TG-3′ (expected product 540 bp). RT-PCR conditions were optimized so that the gene products were obtained during the exponential phase of the amplification. Amplification products were fractionated on 1.6% (w/v) agarose gels and visualized by ethidium bromide.

ChIP analysis

ChIP analysis was performed on T98G and U87 cells at 80% confluency following the Upstate ChIP Assay Kit protocol (cat #17-295). Briefly, cells were treated for 24 h in serum-free media with or without IL-6 (100 ng/mL) and quercetin (25 μM). For the immunoprecipitation, 4 μg of the anti-STAT3 or non-specific IgG were used for each condition and incubated with the cross-linked complexes overnight at 4 °C. PCR of the human *cyclin D1* promoter on the input and isolated DNA was performed using Platinum® *Taq* DNA Polymerase (Invitrogen; Burlington, ON). The *cyclin D1* promoter primer sequences were as follows: forward, 5′-GAT TTT CTT TCA AAC AAC GTG GTT AC-3′, and reverse 5′-TCT TGG TGA CCA TTT GGA GAC A-3′ (expected product 92 bp) [30].

Migration assays

U87 and T98G cell migration was performed using Transwell filters (8-μm pore size; Costar, Cambridge, MA) precoated with 0.15% gelatin for 24 h at 4 °C. The Transwells were then washed once with PBS and assembled into 24-well plates. The upper chamber of each Transwell was filled with 1.0×10^5 cells (100 μL) in serum-free medium and cells were allowed to adhere for 30 min. Then, fresh medium (10% BCS) containing vehicle or various concentrations of quercetin and IL-6 was placed in the lower wells. One hundred microliters of two-fold concentrated vehicle, drug or IL-6 solution prepared in serum-free medium was loaded into each of the upper wells. The plate was placed at 37 °C in 5% CO_2/95% air for 16–18 h. For all experiments, cells that had migrated to the lower surface of the filters were fixed with 10% formalin phosphate and stained with 0.1% Crystal Violet/20% methanol. Cell migration was quantified by computer-assisted imaging using Northern Eclipse software (Empix Imaging, Mississauga, ON) and data were expressed as the average density of migrated cells per four fields (magnification 50×).

Gelatin zymography

Gelatin zymography was used to assess the extent of proMMP-2 activity as previously described [31]. Briefly, an aliquot (20 μl) of the culture medium was subjected to SDS-PAGE in a gel containing 0.1 mg/mL gelatin. The gels were then incubated in 2.5% Triton X-100 and rinsed in nanopure distilled H_2O. Gels were further incubated at 37 °C for 20 h in 20 mM NaCl, 5 mM $CaCl_2$, 0.02% Brij-35, 50 mM Tris–HCl buffer, pH 7.6, then stained with 0.1% Coomassie Brilliant blue R-250 and destained in 10% acetic acid, 30% methanol in H_2O. Gelatinolytic activity was detected as unstained bands on a blue background.

Data statistical analysis

Statistical analyses were performed with Student's *t*-test when one group was compared with the control group. To compare amongst two or more groups, one-way analysis of variance

(ANOVA) and Dunnett's multiple comparison post hoc test were used. All statistical analyses were performed using GraphPad Prism version 5.0b for Macintosh (GraphPad Software, San Diego, CA, USA; www.graphpad.com). Significance was assumed for P values less than 0.05.

Results

The IL-6/STAT3 signaling pathway in human glioblastoma cell lines

In order to evaluate the effect of quercetin on the signaling pathway regulated by IL-6, we first characterized the IL-6 receptor complex by Western blot in different human glioblastoma cell lines. All of the glioblastoma cell lines tested expressed the IL-6Rα and GP130 receptors (Fig. 1). Among the cell lines evaluated, T98G and U87 expressed the highest levels of both IL-6 receptors (IL-6Rα and GP130). Thus, T98G and U87 human glioblastoma cell lines provide a suitable cellular model for studying pharmacological inhibition of the IL-6/STAT3 signaling pathway.

Quercetin inhibits IL-6-induced JAK/STAT3 signaling pathway

Diet-derived flavonoids have the ability to affect various growth factor and cytokine networks, including VEGF/VEGFR2, HGF/Met and PDGF/PDGFR signaling [32–37]. To evaluate whether quercetin (see molecular structure in Fig. 2A) can also inhibit the JAK/STAT3 signaling pathway, we first evaluated the capacity of quercetin to inhibit STAT3 constitutive phosphorylation (Fig. 2B). T98G and U87 cells were incubated for 18 h in serum-free medium in the presence or absence of quercetin, and baseline STAT3 phosphorylation were immunodetected. Under our conditions, we observed that STAT3 was constitutively activated in both T98G and U87 cells. Quercetin alone did not induce noticeable changes in phosphorylated STAT3 basal level indicating that quercetin unaffected STAT3 constitutive phosphorylation. Secondly, we examined the possibility that quercetin could affect the JAK/STAT3 signaling pathway induced by IL-6 stimulation in T98G and U87 glioblastoma cells (Figs. 2C–D). Cells were incubated for 18 h in serum-free medium in the presence or absence of various quercetin concentrations and the cells were then stimulated with IL-6 (100 ng/mL) for 15 min, following which GP130 serine782 phosphorylation status was assessed (Figs. 2C–D). We found that IL-6 induced a remarkable increase in GP130, JAK1, JAK2 and STAT3 phosphorylation. Quercetin inhibited GP130 phosphorylation induced by IL-6 in a dose-dependent manner with IC_{50} values of ~30 μM and ~18 μM for T98G and U87 cells, respectively (Figs. 2C–D). Moreover, quercetin also inhibited JAK1 phosphorylation with IC_{50} values of ~17 μM and ~3 μM for T98G and U87 cells, respectively (Figs. 2C–D). Also, as shown in Figs. 2C–D, the inhibition of IL-6-induced phosphorylation of STAT3 by quercetin was dependent on the concentration, with an IC_{50} of ~20 μM for both T98G (Fig. 2C) and U87 (Fig. 2D) glioblastoma cells. JAK2 phosphorylation induced by IL-6 was not significantly affected by quercetin in either T98G or U87 glioblastoma cells (Figs. 2C–D), and GAPDH expression was used as an internal loading control.

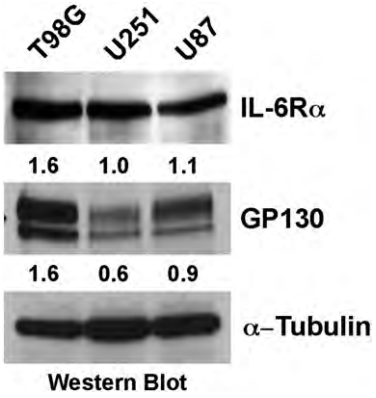

Fig. 1 – Expression of IL-6 receptors in human glioblastoma cell lines. The IL-6Rα and GP130 receptors were immunodetected in cell lysates (20 μg of protein) from glioblastoma cell lines using specific antibodies, as described in Materials and methods. The band intensity was quantified with ImageJ software (Wayne Rasband, NIH) and expressed in arbitrary units as a ratio of the levels of IL-6Rα and GP130 to those of α-tubulin to correct for variations in the amounts of protein.

Quercetin inhibits the growth of glioblastoma cells induced by IL-6

Thymidine incorporation assays were performed to determine whether quercetin affected IL-6-induced cell proliferation *in vitro* (Figs. 3A–B). We showed that IL-6 (100 ng/mL) significantly stimulated the proliferation of T98G and U87 cells after 48 h incubation (Fig. 3A). Exposure of glioblastoma cells for 48 h to quercetin in the presence of IL-6 suppressed the IL-6-induced cell growth in a concentration-dependent manner, with IC_{50} values of 17.1 μM and 24.3 μM in U87 and T98G glioblastoma cells, respectively (Fig. 3B). The effect of quercetin on cell proliferation induced by IL-6 was also investigated by flow cytometry in PI-stained cells (Fig. 3C; quantification *right panels*). T98G and U87 cells were incubated for 24 h in serum-free medium in the presence or absence of IL-6 (100 ng/mL) and 25 μM of quercetin. When deprived of serum, T98G and U87 cells entered a viable G_1 arrested state, as observed in the control condition. In the presence of IL-6, the cell population in the proliferative ($S+G_2/M$) phases of the cell cycle significantly increased in a similar manner in both cell lines, whereas the addition of quercetin completely antagonized this effect (Fig. 3C). In a cell population, cell growth is the process deriving from the balance of cell proliferation and cell death. Under the conditions used here, quercetin (up to 100 μM) did not significantly increase either apoptotic or necrotic T98G (Fig. 3D) or U87 (Fig. 3E) cell populations in the presence of IL-6, as assessed by PI/Annexin V staining and flow cytometry analysis. Thus, the suppression of glioblastoma cell growth by quercetin was not due to apoptosis or cellular toxicity. Collectively, these data suggest that STAT3 activation is involved in glioblastoma cell growth regulation and that inhibition of STAT3 activation by quercetin induces cell cycle arrest and suppresses glioblastoma cell proliferation.

Quercetin inhibits Rb protein phosphorylation

We examined the effect of quercetin on IL-6-stimulated cells by western blotting analysis of serine phosphorylation sites at serine795 and 780 of the Rb protein (Fig. 4). In the presence of

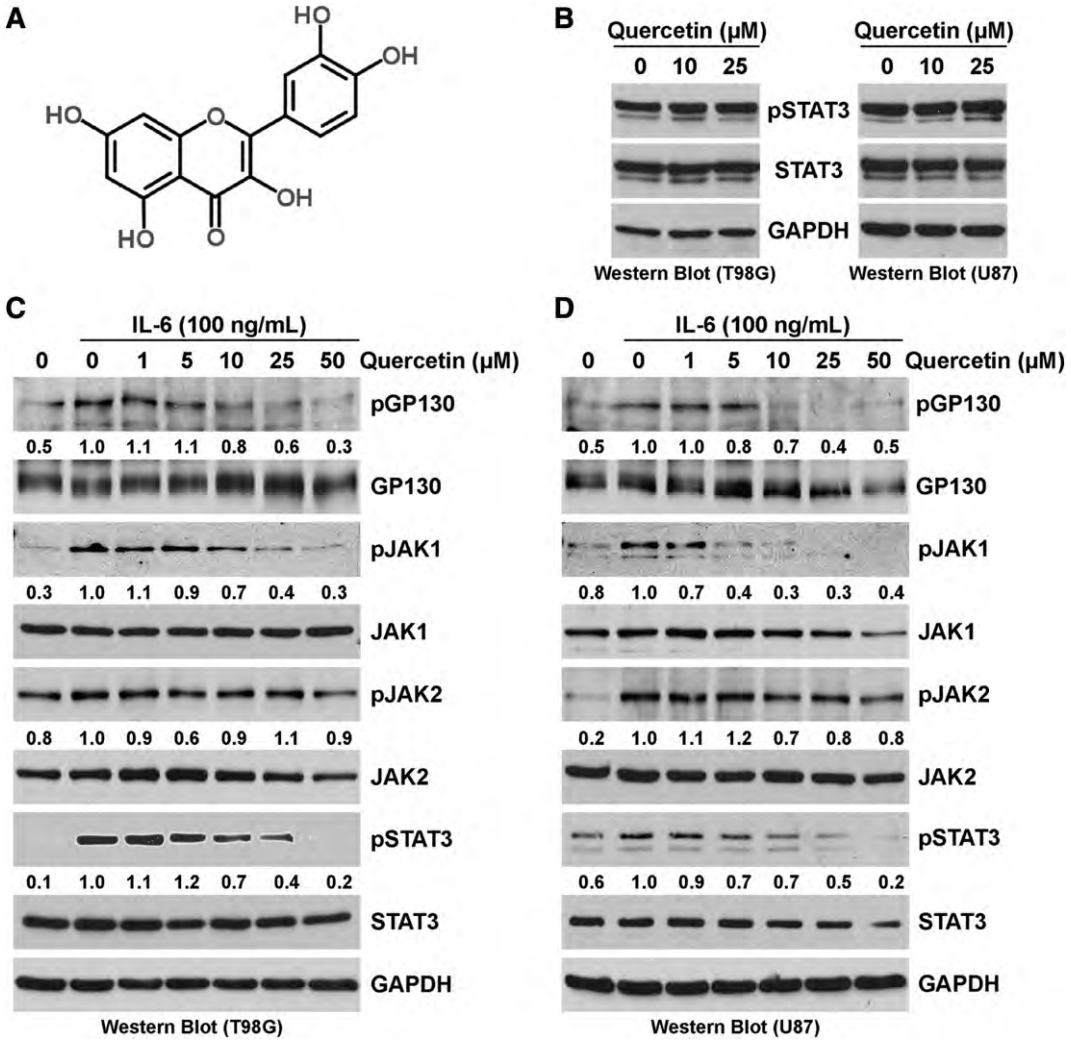

Fig. 2 – Quercetin inhibits IL-6-induced JAK/STAT3 signaling pathway. (A) Chemical structure of quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one). (B) T98G and U87 cells were incubated in serum-free media for 18 h in the presence of various quercetin concentrations. T98G (C) and U87 (D) cells were incubated in serum-free media for 18 h in the presence of various quercetin concentrations. Then, to study cell signaling, the cells were stimulated with 100 ng/mL IL-6 for 15 min, as described in Materials and methods. After cell treatments, the extent of GP130 serine782 phosphorylation, JAK1 tyrosine1022/1023 phosphorylation, JAK2 tyrosine1007/1008 phosphorylation, STAT3 tyrosine705 phosphorylation, along with their total protein level and GAPDH were monitored by immunoblotting using specific antibodies. Data are representative of three independent experiments. Immunodetection obtained from representative samples are shown. The band intensity was quantified with ImageJ software and expressed in arbitrary units as a ratio of levels of phosphorylated protein to those of the total protein to correct for variation in the amount of protein. The relative levels of phosphorylated protein were also normalized to the control (value = 1).

IL-6, the total Rb protein remained constant, whereas Rb phosphorylation was increased (Figs. 4A–B). Moreover, quercetin treatment inhibited Rb phosphorylation levels at serines 795 and 780 in a dose-dependent manner, with an IC_{50} ~5–10 μM for both T98G (Fig. 4A) and U87 (Fig. 4B) glioblastoma cells. These results suggest that the G_0/G_1 cell cycle arrest observed with quercetin in the presence of IL-6 could be related to the inhibition of Rb phosphorylation.

Quercetin inhibits cyclin D1 expression

We investigated whether the expression of cyclin D1 was modulated by quercetin in order to determine the underlying mechanism of IL-6-induced glioblastoma cell proliferation inhibition by quercetin (Fig. 5). As observed by Western blot, cyclin D1 is not significantly affected by quercetin in the absence of IL-6 (Figs. 5A–B), whereas quercetin treatment in the presence of IL-6 strongly decreased the protein level of cyclin D1 in a dose-dependent manner in both glioblastoma cell lines (Figs. 5C–D). Also, under our conditions, the protein level of cdk4 was not significantly affected by IL-6 and quercetin (Figs. 5C–D). Since quercetin inhibits cyclin D1 expression in the presence of IL-6, mRNA expression of cyclin D1 was estimated by RT-PCR in T98G (Fig. 5E) and U87 (Fig. 5F) cells following treatment with or without IL-6 (100 ng/mL) and quercetin (25 μM). Quercetin significantly reduced cyclin D1 mRNA in the presence of IL-6 compared to control cells, whereas cyclin D1 mRNA was

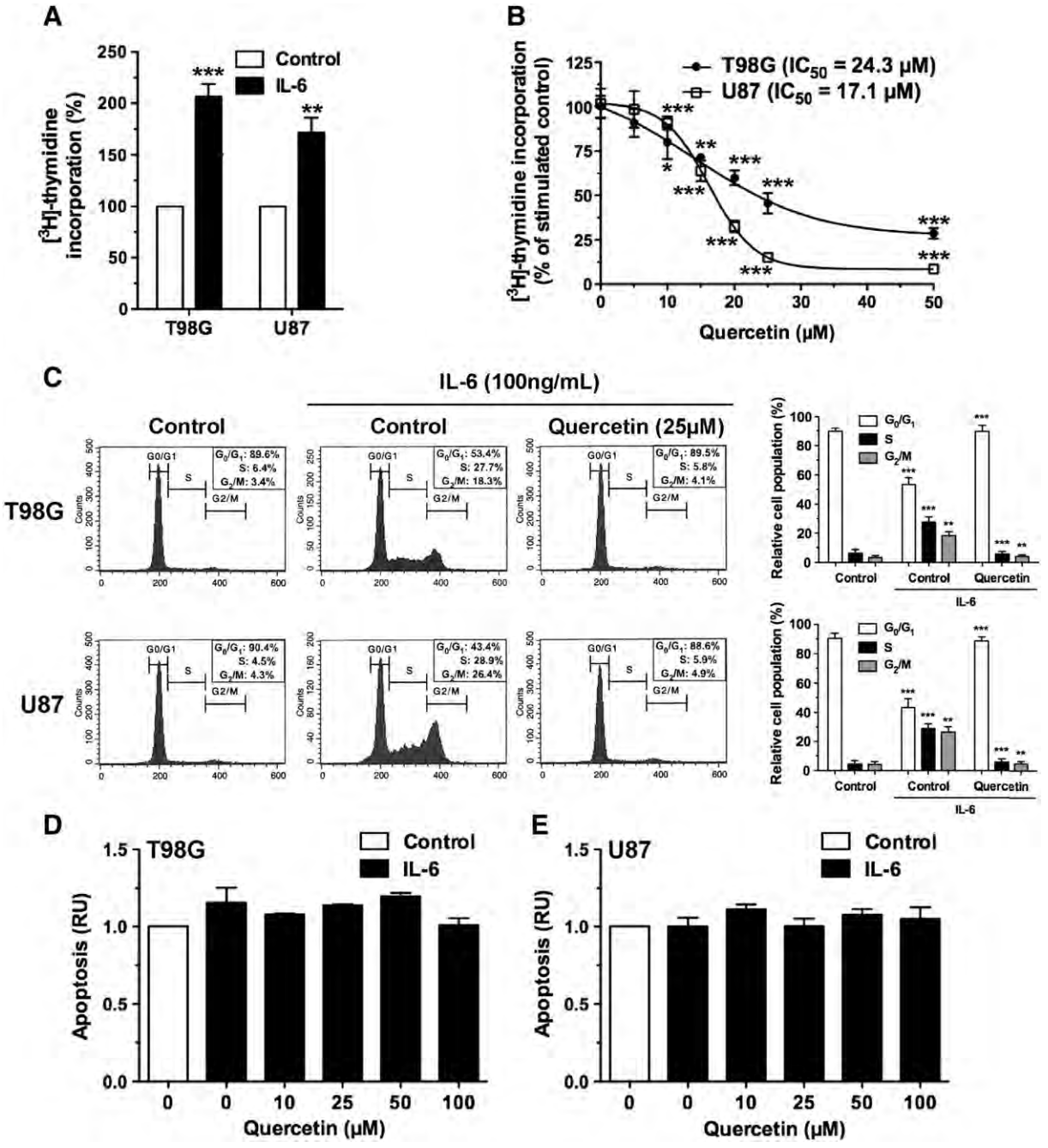

Fig. 3 – Quercetin inhibits the growth of glioblastoma cells induced by IL-6. T98G and U87 cells were seeded onto 96-well plates. After 24 h, cells were starved in serum-free media for 20 h. Quiescent cells were then treated for 48 h with or without IL-6 (100 ng/mL) (A), or with various concentrations of quercetin in the presence of IL-6 (100 ng/mL) (B). Then 0.5 μCi/well of [^{3}H]-thymidine was added and the amount of [^{3}H]-thymidine incorporated into the cells was measured as described in Materials and methods. Data represent the means ± standard error (SE) of results obtained from three different experiments. (C) Representative flow cytometry profiles of T98G (*upper panel*) and U87 (*lower panel*) cells in the G_0/G_1, S and G_2/M phases as determined by flow cytometric analysis. The individual DNA content was determined by fluorescence intensity of incorporated propidium iodide, as described in Materials and methods. Cell population profiles obtained from representative samples are shown (*Left panel*), and data represent the means ± SE of results obtained from three different experiments (*Right panel*). Analysis of annexin V-FITC and propidium iodide (PI) staining in T98G (D) and U87 (E) cells, as described in Materials and methods. Results were normalized to the control without IL-6 (value = 1), and represent cells that are positive for apoptosis (FITC-Annexin V positive, PI negative or FITC-Annexin V positive, PI positive) compared to the control without IL-6. Data represent the means ± SE of results obtained from three different experiments. Statistically significant differences, as compared with control conditions are indicated as follows: $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ (A, Student's *t*-test; B, *ANOVA*).

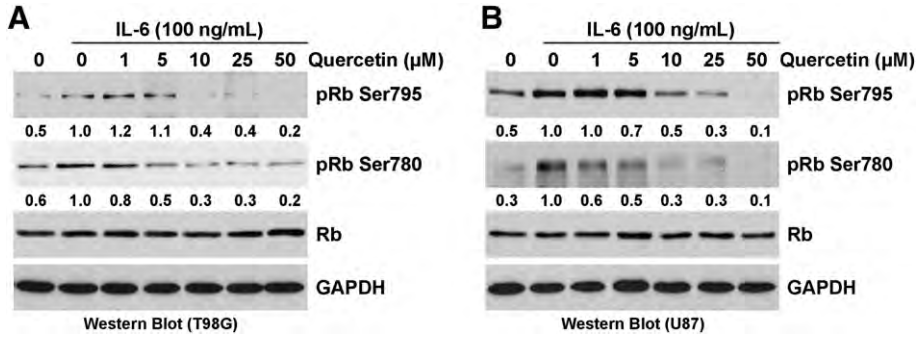

Fig. 4 – Quercetin inhibits Rb protein phosphorylation. T98G (A) and U87 (B) cells were treated with or without IL-6 (100 ng/mL) and various concentrations of quercetin for 18 h, as described in Materials and methods. The extent of Rb serine795 and serine780 phosphorylation, along with the total Rb protein level and GAPDH were monitored by immunoblotting using specific antibodies. Immunodetection obtained from representative samples are shown, and data are representative of three independent experiments. The band intensity was quantified with ImageJ software and expressed in arbitrary units as a ratio of levels of phosphorylated protein to those of the total protein to correct for variation in the amount of protein. The relative levels of phosphorylated protein were also normalized to the control (value = 1).

unaffected by quercetin in the absence of IL-6. We next examined whether the effect of quercetin on cyclin D1 protein and mRNA levels is mediated by reduced recruitment of the STAT3 transcription factor at the *cyclin D1* promoter in the presence of IL-6 (Fig. 5G). By ChIP analysis, we first observed that STAT3 was indeed recruited to the *cyclin D1* promoter in the presence of IL-6 (Fig. 5G; *right panel*). In quercetin-treated cells, we showed a decrease in physical association of STAT3 at the *cyclin D1* locus when IL-6 is present (Fig. 5G). These findings suggest that quercetin regulates cyclin D1 expression by regulating the recruitment of STAT3 transcription factor at the *cyclin D1* promoter. Since cyclin D and cdk4 complex [24] phosphorylate Ser780 position of Rb protein, the decrease in the phosphorylation level of Rb protein (Figs. 4A–B) appeared to be mediated by the suppression of cyclin D1 protein expression.

Quercetin inhibits IL-6-induced MMP2 secretion and migration of glioblastoma cells

Since quercetin inhibits STAT3 activation induced by IL-6 (Fig. 2), and that STAT3 directly regulates MMP-2 expression and cancer cell migration [15,25–27], we evaluated the effect of quercetin on the IL-6-mediated secretion of MMP-2 and cell migration (Fig. 6). The level of proMMP2 gelatinolytic activity in the supernatant was assessed by gelatin zymography in T98G and U87 glioblastoma cells after treatment with or without IL-6 (100 ng/mL) and various quercetin concentrations. IL-6 increased the cellular secretion of proMMP-2 and quercetin antagonized this effect in a dose-responsive manner (Fig. 6A). We next examined the effect of quercetin on glioblastoma cell migration regulated by IL-6. Evaluated by the Transwell chamber system, T98G and U87 cells were allowed to adhere to gelatin-coated Transwells and were incubated for 18 h with various concentrations of IL-6 in the lower chamber (Fig. 6B). Under these conditions, we found that IL-6 significantly promoted T98G and U87 cell migration in a dose-dependent manner (Fig. 6B). We then evaluated the effect of quercetin on IL-6-induced glioblastoma cell migration using various concentrations of quercetin in the presence of IL-6 (100 ng/mL). Under these conditions, quercetin inhibited IL-6 induced migration of T98G and U87 cells in a dose-dependent manner (Fig. 6C), with IC_{50} ranging from 14.6 μM for U87 to 22.7 μM for T98G. Altogether, these results suggest that quercetin could inhibit glioblastoma cell migration by reducing the MMP-2 secretion regulated by IL-6.

Discussion

STAT3, a member of the STAT family of transcription factors, is a key player involved in cancer-related inflammation. STAT3 is frequently deregulated in many types of cancer and functions as an oncogene during tumorigenesis [29]. Activation of STAT3 results in the expression of downstream genes that control major cellular responses (including cell proliferation and invasion as well as cancer cell survival) such as cyclin D1, MMP2 and Bcl-2 [38]. The crucial role of STAT3 in cancer progression and tumorigenesis has allowed STAT3 to emerge as a promising molecular target for treating cancer. The goal of the present study was to characterize whether quercetin exerts any anticancer effects through abrogation of the IL-6/STAT3 signaling pathway in glioblastoma cells.

In the present study, we found that quercetin inhibited IL-6-inducible GP130 phosphorylation in glioblastoma cells in parallel with the suppression of JAK1 activation, whereas quercetin has no significant effect on JAK2. Also, unlike curcumin [39,40] and EGCG [41], two phytochemical inhibitors of the constitutive and the IL-6-inducible STAT3 signaling pathway, quercetin inhibits the phosphorylation of STAT3 induced by IL-6 without affecting the constitutive baseline level of phosphorylated STAT3. Also, the effective dose of quercetin used to inhibits IL-6 signaling are consistent with those obtained with curcumin and EGCG. As previously reported among glioblastoma cell lines, STAT3 is constitutively activated at various levels [16], which could explain the difference in the baseline STAT3 phosphorylation between T98G and U87 cells. Moreover, quercetin down-regulated the expression of STAT3-regulated target gene cyclin D1 and the secretion of MMP-2 mediated by IL-6 signaling, and antagonized both the stimulative effect of IL-6 on cell proliferation and migration as well as the IL-6-induced accumulation of cells in the proliferative ($S + G_2/M$) phases of the cell cycle. Some slight differences were also observed among the cells tested in the inhibitory effect of quercetin on cell proliferation stimulated by

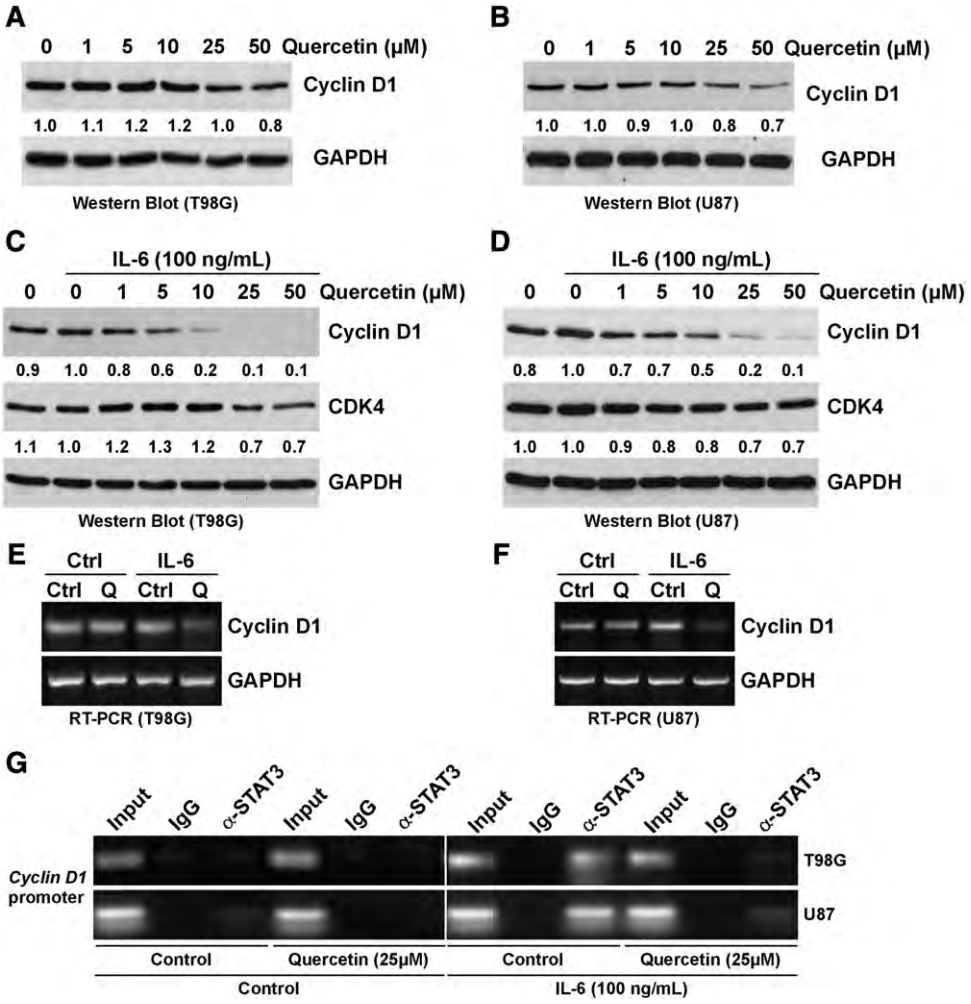

Fig. 5 – Quercetin inhibits cyclin D1 expression. T98G (A, C, E) and U87 (B, D, F) cells were treated with or without IL-6 (100 ng/mL) and various concentrations of quercetin for 18 h. The levels of cyclin D1, CDK4 and GAPDH were monitored by immunoblotting using specific antibodies (A–D), and the levels of cyclin D1 and GAPDH mRNA by RT-PCR (E–F). Immunodetection and amplification obtained from representative samples are shown, and data are representative of three independent experiments. The band intensity was quantified with ImageJ software and expressed in arbitrary units as a ratio of levels of cyclin D1 or CDK4 to those of GAPDH to correct for variations in the amount of protein. The relative levels of phosphorylated protein were also normalized to the control (value = 1). (G) Analysis of the occupancy of STAT3 transcription factor at the *cyclin D1* gene promoter by ChIP analysis. ChIP assays were performed with chromatin prepared from T98G (*upper panel*) and U87 (*lower panel*) cells treated with or without quercetin (25 μM) and IL-6 (100 ng/mL), as described in Materials and methods. Data are representative of two independent experiments. PCR results obtained from representative samples are shown.

IL-6. These observations could be explained in part by intercellular differences in the dependency of the cells from other proteins known to affect the IL-6/JAK/STAT3 pathway, e.g. suppressors of cytokine signaling 3 (SOCS3) [42], protein inhibitor of activated STAT (PIAS) [43], and non-receptor tyrosine kinases, such as Src [44] and BMX [45].

The tyrosine kinase Hck [46] and the phosphoinositide 3-kinase (PI3K) [47] have previously been crystallized with quercetin. Relative to both Hck and PI3K kinase/quercetin complex interaction, we hypothesize as a possible molecular mechanism of action that quercetin may bind to the GP130, STAT3 or JAK1 putative ATP binding sites via hydrophobic and ionic interactions. This interaction could subsequently abrogate their phosphorylation/activation, and in turn inhibit the stimulatory action of IL-6 on cell proliferation, cell cycle progression, cell migration and MMP-2 secretion. However, at any given time, the total STAT3 activity levels in a cell are the sum of the effects of cell to cell adhesion plus the conventional STAT3 activating/inhibiting factors present [48,49]. Thus, we cannot exclude the possibility that quercetin could also modulate other mechanisms that regulate cell response to IL-6 and STAT3 activity. This is the first report that documents quercetin as a novel pharmacological blocker of the IL-6/STAT3 signaling pathway in glioblastoma cells, and provided further evidence of the molecular mechanism underlying the anticancer activity of quercetin.

Under the conditions used here, quercetin antagonized the proliferative effect of IL-6 in glioblastoma cells, as evidenced by the decreased cyclin D1 and Rb phosphorylation. Phosphorylation of Rb plays a crucial role in the progression of G_1 phase and the transition from G_1 to S phase during cell proliferation. In the hypophosphorylated state, Rb is active and carries out its role as a tumor suppressor

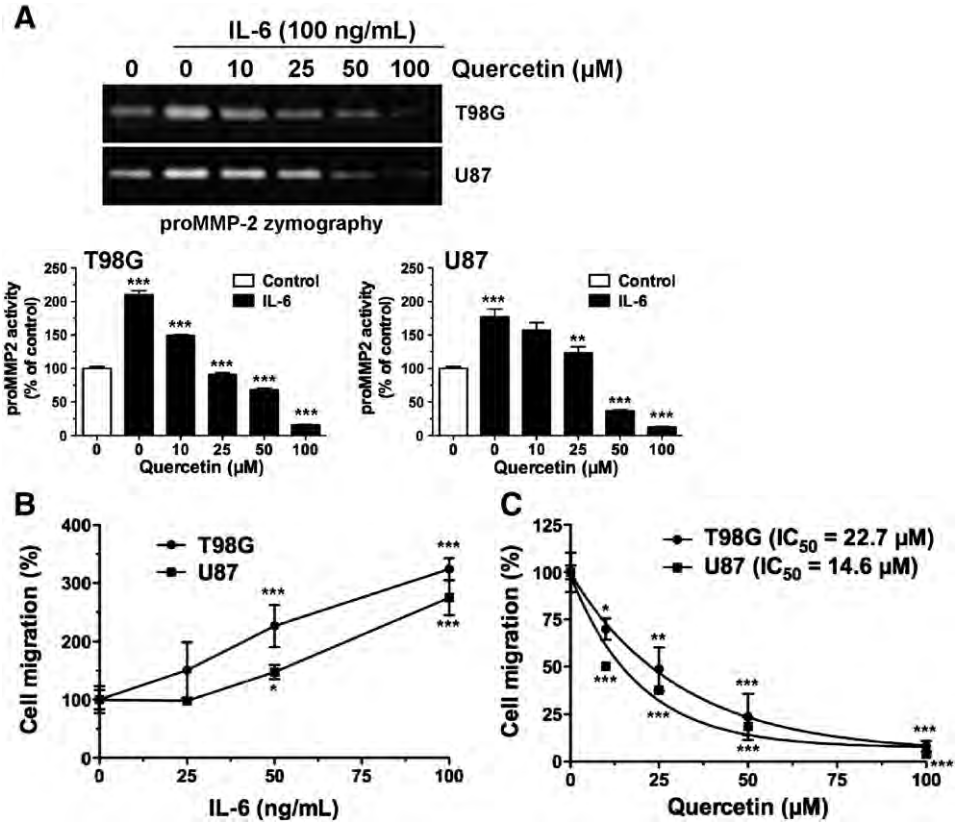

Fig. 6 – Quercetin inhibits glioblastoma MMP-2 secretion and cell migration induced by IL-6. (A) The proMMP-2 gelatinolytic activity was assessed by gelatin zymography in the cell-conditioned media after 24 h of treatment with or without IL-6 (100 ng/mL) and various concentrations of quercetin, as described in Materials and methods. The extent of gelatinolytic activity was quantified by densitometry and expressed as a percentage of the control without IL-6. Results were analyzed by densitometry and data represent the means ± SE of results obtained from three different experiments. T98G and U87 cell migration was performed with or without various concentrations of IL-6 (B) or various concentrations of quercetin in the presence of IL-6 (100 ng/mL) (C) using modified Boyden chambers, as described in Materials and methods. Results are expressed as a percentage of cell migration seen in the control cells (without IL-6 or quercetin). Statistically significant differences, as compared with the respective control conditions, are indicated as follows: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ (*ANOVA*).

by inhibiting cell cycle progression and proliferation [23]. Therefore, the inhibitory effect of quercetin on Rb phosphorylation could be related to lower activity of the cyclin D/CDK4 complex due to decreasing levels of cyclin D1. In addition, ChIP analysis of STAT3 demonstrated decreased physical association of STAT3 at the *cyclin D1* locus in quercetin-treated cells, resulting in a reduction of cyclin D1 mRNA and protein. These data suggest that quercetin caused the suppression of cyclin D1 protein expression through transcriptional repression via the reduction of STAT3 recruitment at the *cyclin D1* promoter in the presence of IL-6. But we cannot exclude the possibility that quercetin could also decrease the cyclin D1 protein level through the promotion of proteasomal degradation. Also, in the presence of IL-6, quercetin does not have a cytotoxic effect up to a concentration of 100 μM. Thus, the antiproliferative effect of quercetin may not be due to apoptosis or necrosis in T98G and U87 glioblastoma cells but rather to the inhibition of cell cycle progression induced by the IL-6 cytokine. The present result is in agreement with another study from Siegelin et al. [50] where they showed that quercetin did not inhibit cell viability *in vitro* using various glioblastoma cell lines, including U87 cells. However, these results seem to be cell type-specific since quercetin could reduce cell viability in A172 glioblastoma cells [51]. Altogether, quercetin-induced G_0/G_1 cell cycle arrest is correlated with the suppression of STAT3 activation and cyclin D1 protein levels, as well as the inhibition of Rb phosphorylation in glioblastoma cells. From these observations, it is tempting to speculate that its effects on the Rb pathway could mediate the anti-proliferative activity of quercetin.

In glioblastoma cells, high secretion of MMP-2 can be attributed to elevated STAT3 activity, which upregulates the transcription of MMP-2 through direct interaction of STAT3 with the *MMP-2* promoter [15]. These processes represent crucial events in tumor invasion and the development of metastasis [15,52,53]. Under our conditions, quercetin antagonized the IL-6-induced secretion of MMP-2 as well as glioblastoma cell migration. Thus, targeted blockade of the STAT3 signaling pathway using quercetin represents a potential anti-glioma therapeutic approach. Interestingly, it has also been reported that cyclin D1, a STAT3 target gene, plays a positive role in the induction of cell migration [54,55]. This observation indicates that the reduction of cyclin D1 protein level by quercetin, as a result of the inhibition of the STAT3 signaling induced by IL-6, could also be involved in the repression of glioblastoma cell migration.

According to the World Cancer Research Fund/American Institute for Cancer Food Research, a diet rich in polyphenols from fruit and vegetables has been proposed as a key lifestyle factor for the prevention of a wide variety of cancer types. In our different assays, the quercetin concentrations required to inhibit STAT3 phosphorylation as well as glioblastoma cell proliferation and migration, i.e. IC_{50} around 10 to 25 μM, are much higher than those achieved in serum after a single oral administration in human studies [56,57]. However, as a 100 mg single dose was found to create a serum concentration of 0.8 μM quercetin [56,57], it can be extrapolated that a 1500 mg daily dose might attain a 10 μM level. Also, since quercetin is absorbed in humans and is slowly eliminated [56,57], the relatively long half-life of quercetin may result in even higher serum concentrations after repeated intake of quercetin throughout the day. Data from animal studies suggest that concentrations of quercetin above 10 μM are attainable with continuous daily oral doses in rats [58]. Moreover, a serum quercetin concentration of 12 μM in humans is achievable after a single intravenous dose of 100 mg [59]. Altogether, these results demonstrated that the serum concentration of quercetin required for the anti-cancer properties observed in the present study could be attained in humans. Following consumption, quercetin is bioavailable to a large variety of tissues, including brain [60,61], and may thus have some chemopreventive effects against brain tumors [62]. On the basis of this favorable bioavailability profile, quercetin appears to be of potential interest for glioblastoma prevention and treatment.

In conclusion, targeted blockade of the oncogenic functions of STAT3 by quercetin represents a new therapeutic potential in the management of tumors with constitutive STAT3 activation, like glioblastoma. In fact, glioblastoma tumors and cell lines contain high levels of activated STAT3 when compared with normal human astrocytes, white matter, and normal tissue adjacent to tumors [63]. As such, inhibition of the STAT3 signaling pathway induced by IL-6 using quercetin represents a prime target in the therapeutic efforts aimed at reducing brain tumor progression.

Acknowledgments

Many thanks to Dr. Borhane Annabi and Dr. Sylvie Lamy for their helpful comments on this manuscript. We also thank Julie Poirier for her technical support. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC; #RGPIN3187) to R.B. R.B. holds both an Institutional Research Chair in Cancer Prevention and Treatment from Université du Québec à Montréal (UQÀM), and the Claude Bertrand Chair in Neurosurgery (CHUM). The authors declare no conflict of interest related to this work.

REFERENCES

[1] R. Beliveau, D. Gingras, Role of nutrition in preventing cancer, Can. Fam. Physician 53 (2007) 1905–1911.
[2] J.V. Formica, W. Regelson, Review of the biology of quercetin and related bioflavonoids, Food. Chem. Toxicol. 33 (1995) 1061–1080.
[3] H. Yao, W. Xu, X. Shi, Z. Zhang, Dietary flavonoids as cancer prevention agents, J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 29 (2011) 1–31.
[4] S. Chirumbolo, The role of quercetin, flavonols and flavones in modulating inflammatory cell function, Inflamm. Allergy Drug Targets 9 (2010) 263–285.
[5] S.C. Bischoff, Quercetin: potentials in the prevention and therapy of disease, Curr. Opin. Clin. Nutr. Metab. Care 11 (2008) 733–740.
[6] A. Murakami, H. Ashida, J. Terao, Multitargeted cancer prevention by quercetin, Cancer Lett. 269 (2008) 315–325.
[7] S.A. Grossman, J.F. Batara, Current management of glioblastoma multiforme, Semin. Oncol. 31 (2004) 635–644.
[8] H. Ohgaki, Epidemiology of brain tumors, Methods Mol. Biol. 472 (2009) 323–342.
[9] D.S. Hong, L.S. Angelo, R. Kurzrock, Interleukin-6 and its receptor in cancer: implications for translational therapeutics, Cancer 110 (2007) 1911–1928.
[10] T. Bowman, R. Garcia, J. Turkson, R. Jove, STATs in oncogenesis, Oncogene 19 (2000) 2474–2488.
[11] C.Y. Chang, M.C. Li, S.L. Liao, Y.L. Huang, C.C. Shen, H.C. Pan, Prognostic and clinical implication of IL-6 expression in glioblastoma multiforme, J. Clin. Neurosci. 12 (2005) 930–933.
[12] E.C. Brantley, E.N. Benveniste, Signal transducer and activator of transcription-3: a molecular hub for signaling pathways in gliomas, Mol. Cancer Res. 6 (2008) 675–684.
[13] T. Hirano, K. Nakajima, M. Hibi, Signaling mechanisms through gp130: a model of the cytokine system, Cytokine Growth Factor Rev. 8 (1997) 241–252.
[14] Q. Liu, G. Li, R. Li, J. Shen, Q. He, L. Deng, C. Zhang, J. Zhang, IL-6 promotion of glioblastoma cell invasion and angiogenesis in U251 and T98G cell lines, J. Neurooncol. 100 (2010) 165–176.
[15] T.X. Xie, D. Wei, M. Liu, A.C. Gao, F. Ali-Osman, R. Sawaya, S. Huang, Stat3 activation regulates the expression of matrix metalloproteinase-2 and tumor invasion and metastasis, Oncogene 23 (2004) 3550–3560.
[16] H. Yu, D. Pardoll, R. Jove, STATs in cancer inflammation and immunity: a leading role for STAT3, Nat. Rev. Cancer 9 (2009) 798–809.
[17] M.M. Brierley, E.N. Fish, Stats: multifaceted regulators of transcription, J. Interferon Cytokine Res. 25 (2005) 733–744.
[18] P. Birner, K. Toumangelova-Uzeir, S. Natchev, M. Guentchev, STAT3 tyrosine phosphorylation influences survival in glioblastoma, J. Neurooncol. 100 (2010) 339–343.
[19] M. Masuda, M. Suzui, R. Yasumatu, T. Nakashima, Y. Kuratomi, K. Azuma, K. Tomita, S. Komiyama, I.B. Weinstein, Constitutive activation of signal transducers and activators of transcription 3 correlates with cyclin D1 overexpression and may provide a novel prognostic marker in head and neck squamous cell carcinoma, Cancer Res. 62 (2002) 3351–3355.
[20] B.B. Aggarwal, A.B. Kunnumakkara, K.B. Harikumar, S.R. Gupta, S.T. Tharakan, C. Koca, S. Dey, B. Sung, Signal transducer and activator of transcription-3, inflammation, and cancer: how intimate is the relationship? Ann. N. Y. Acad. Sci. 1171 (2009) 59–76.
[21] R. Li, G. Li, L. Deng, Q. Liu, J. Dai, J. Shen, J. Zhang, IL-6 augments the invasiveness of U87MG human glioblastoma multiforme cells via up-regulation of MMP-2 and fascin-1, Oncol. Rep. 23 (2010) 1553–1559.
[22] K. Leslie, C. Lang, G. Devgan, J. Azare, M. Berishaj, W. Gerald, Y.B. Kim, K. Paz, J.E. Darnell, C. Albanese, T. Sakamaki, R. Pestell, J. Bromberg, Cyclin D1 is transcriptionally regulated by and required for transformation by activated signal transducer and activator of transcription 3, Cancer Res. 66 (2006) 2544–2552.
[23] C.J. Sherr, G1 phase progression: cycling on cue, Cell 79 (1994) 551–555.
[24] M. Kitagawa, H. Higashi, H.K. Jung, I. Suzuki-Takahashi, M. Ikeda, K. Tamai, J. Kato, K. Segawa, E. Yoshida, S. Nishimura, Y. Taya, The consensus motif for phosphorylation by cyclin D1-Cdk4 is different from that for phosphorylation by cyclin A/E-Cdk2, EMBO J. 15 (1996) 7060–7069.
[25] S.H. Kang, M.O. Yu, K.J. Park, S.G. Chi, D.H. Park, Y.G. Chung, Activated STAT3 regulates hypoxia-induced angiogenesis and cell

migration in human glioblastoma, Neurosurgery 67 (2010) 1386–1395 discussion 1395.
[26] C. Senft, M. Priester, M. Polacin, K. Schroder, V. Seifert, D. Kogel, J. Weissenberger, Inhibition of the JAK-2/STAT3 signaling pathway impedes the migratory and invasive potential of human glioblastoma cells, J. Neurooncol. 101 (2011) 393–403.
[27] T.X. Xie, F.J. Huang, K.D. Aldape, S.H. Kang, M. Liu, J.E. Gershenwald, K. Xie, R. Sawaya, S. Huang, Activation of stat3 in human melanoma promotes brain metastasis, Cancer Res. 66 (2006) 3188–3196.
[28] J. Bromberg, J.E. Darnell Jr., The role of STATs in transcriptional control and their impact on cellular function, Oncogene 19 (2000) 2468–2473.
[29] J.F. Bromberg, M.H. Wrzeszczynska, G. Devgan, Y. Zhao, R.G. Pestell, C. Albanese, J.E. Darnell Jr., Stat3 as an oncogene, Cell 98 (1999) 295–303.
[30] A.N. Iberg, A. Espejo, D. Cheng, D. Kim, J. Michaud-Levesque, S. Richard, M.T. Bedford, Arginine methylation of the histone H3 tail impedes effector binding, J. Biol. Chem. 283 (2008) 3006–3010.
[31] A. Sina, S. Lord-Dufour, B. Annabi, Cell-based evidence for aminopeptidase N/CD13 inhibitor actinonin targeting of MT1-MMP-mediated proMMP-2 activation, Cancer Lett. 279 (2009) 171–176.
[32] D. Labbe, M. Provencal, S. Lamy, D. Boivin, D. Gingras, R. Beliveau, The flavonols quercetin, kaempferol, and myricetin inhibit hepatocyte growth factor-induced medulloblastoma cell migration, J. Nutr. 139 (2009) 646–652.
[33] L. Labrecque, S. Lamy, A. Chapus, S. Mihoubi, Y. Durocher, B. Cass, M.W. Bojanowski, D. Gingras, R. Beliveau, Combined inhibition of PDGF and VEGF receptors by ellagic acid, a dietary-derived phenolic compound, Carcinogenesis 26 (2005) 821–826.
[34] S. Lamy, E. Beaulieu, D. Labbe, V. Bedard, A. Moghrabi, S. Barrette, D. Gingras, R. Beliveau, Delphinidin, a dietary anthocyanidin, inhibits platelet-derived growth factor ligand/receptor (PDGF/PDGFR) signaling, Carcinogenesis 29 (2008) 1033–1041.
[35] S. Lamy, V. Bedard, D. Labbe, H. Sartelet, C. Barthomeuf, D. Gingras, R. Beliveau, The dietary flavones apigenin and luteolin impair smooth muscle cell migration and VEGF expression through inhibition of PDGFR-beta phosphorylation, Cancer Prev. Res. (Phila) 1 (2008) 452–459.
[36] S. Lamy, M. Blanchette, J. Michaud-Levesque, R. Lafleur, Y. Durocher, A. Moghrabi, S. Barrette, D. Gingras, R. Beliveau, Delphinidin, a dietary anthocyanidin, inhibits vascular endothelial growth factor receptor-2 phosphorylation, Carcinogenesis 27 (2006) 989–996.
[37] S. Lamy, D. Gingras, R. Beliveau, Green tea catechins inhibit vascular endothelial growth factor receptor phosphorylation, Cancer Res. 62 (2002) 381–385.
[38] J.V. Alvarez, D.A. Frank, Genome-wide analysis of STAT target genes: elucidating the mechanism of STAT-mediated oncogenesis, Cancer Biol. Ther. 3 (2004) 1045–1050.
[39] A.C. Bharti, N. Donato, B.B. Aggarwal, Curcumin (diferuloylmethane) inhibits constitutive and IL-6-inducible STAT3 phosphorylation in human multiple myeloma cells, J. Immunol. 171 (2003) 3863–3871.
[40] R. Blasius, S. Reuter, E. Henry, M. Dicato, M. Diederich, Curcumin regulates signal transducer and activator of transcription (STAT) expression in K562 cells, Biochem. Pharmacol. 72 (2006) 1547–1554.
[41] B.H. Zhu, H.Y. Chen, W.H. Zhan, C.Y. Wang, S.R. Cai, Z. Wang, C.H. Zhang, Y.L. He, (−)-Epigallocatechin-3-gallate inhibits VEGF expression induced by IL-6 via Stat3 in gastric cancer, World J. Gastroenterol. 17 (2011) 2315–2325.
[42] H.A. Bluyssen, M.M. Rastmanesh, C. Tilburgs, K. Jie, S. Wesseling, M.J. Goumans, P. Boer, J.A. Joles, B. Braam, IFN gamma-dependent SOCS3 expression inhibits IL-6-induced STAT3 phosphorylation and differentially affects IL-6 mediated transcriptional responses in endothelial cells, Am. J. Physiol. Cell Physiol. 299 (2010) C354–C362.
[43] E.C. Brantley, L.B. Nabors, G.Y. Gillespie, Y.H. Choi, C.A. Palmer, K. Harrison, K. Roarty, E.N. Benveniste, Loss of protein inhibitors of activated STAT-3 expression in glioblastoma multiforme tumors: implications for STAT-3 activation and gene expression, Clin. Cancer Res. 14 (2008) 4694–4704.
[44] R. Garcia, T.L. Bowman, G. Niu, H. Yu, S. Minton, C.A. Muro-Cacho, C.E. Cox, R. Falcone, R. Fairclough, S. Parsons, A. Laudano, A. Gazit, A. Levitzki, A. Kraker, R. Jove, Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells, Oncogene 20 (2001) 2499–2513.
[45] O.A. Guryanova, Q. Wu, L. Cheng, J.D. Lathia, Z. Huang, J. Yang, J. MacSwords, C.E. Eyler, R.E. McLendon, J.M. Heddleston, W. Shou, D. Hambardzumyan, J. Lee, A.B. Hjelmeland, A.E. Sloan, M. Bredel, G.R. Stark, J.N. Rich, S. Bao, Nonreceptor tyrosine kinase BMX maintains self-renewal and tumorigenic potential of glioblastoma stem cells by activating STAT3, Cancer Cell 19 (2011) 498–511.
[46] F. Sicheri, I. Moarefi, J. Kuriyan, Crystal structure of the Src family tyrosine kinase Hck, Nature 385 (1997) 602–609.
[47] E.H. Walker, M.E. Pacold, O. Perisic, L. Stephens, P.T. Hawkins, M.P. Wymann, R.L. Williams, Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine, Mol Cell 6 (2000) 909–919.
[48] R. Arulanandam, M. Geletu, H. Feracci, L. Raptis, Activated Rac1 requires gp130 for Stat3 activation, cell proliferation and migration, Exp. Cell Res. 316 (2010) 875–886.
[49] L. Raptis, R. Arulanandam, A. Vultur, M. Geletu, S. Chevalier, H. Feracci, Beyond structure, to survival: activation of Stat3 by cadherin engagement, Biochem. Cell Biol. 87 (2009) 835–843.
[50] M.D. Siegelin, D.E. Reuss, A. Habel, A. Rami, A. von Deimling, Quercetin promotes degradation of survivin and thereby enhances death-receptor-mediated apoptosis in glioma cells, Neuro Oncol. 11 (2009) 122–131.
[51] E.J. Kim, C.H. Choi, J.Y. Park, S.K. Kang, Y.K. Kim, Underlying mechanism of quercetin-induced cell death in human glioma cells, Neurochem. Res. 33 (2008) 971–979.
[52] J. Bromberg, X. Chen, STAT proteins: signal tranducers and activators of transcription, Methods Enzymol. 333 (2001) 138–151.
[53] D.E. Levy, C.K. Lee, What does Stat3 do? J. Clin. Invest. 109 (2002) 1143–1148.
[54] Z. Li, X. Jiao, C. Wang, X. Ju, Y. Lu, L. Yuan, M.P. Lisanti, S. Katiyar, R.G. Pestell, Cyclin D1 induction of cellular migration requires p27(KIP1), Cancer Res. 66 (2006) 9986–9994.
[55] Z. Li, C. Wang, G.C. Prendergast, R.G. Pestell, Cyclin D1 functions in cell migration, Cell Cycle 5 (2006) 2440–2442.
[56] P.C. Hollman, M. vd Gaag, M.J. Mengelers, J.M. van Trijp, J.H. de Vries, M.B. Katan, Absorption and disposition kinetics of the dietary antioxidant quercetin in man, Free Radic. Biol. Med. 21 (1996) 703–707.
[57] P.C. Hollman, J.M. van Trijp, M.J. Mengelers, J.H. de Vries, M.B. Katan, Bioavailability of the dietary antioxidant flavonol quercetin in man, Cancer Lett. 114 (1997) 139–140.
[58] C. Morand, V. Crespy, C. Manach, C. Besson, C. Demigne, C. Remesy, Plasma metabolites of quercetin and their antioxidant properties, Am. J. Physiol. 275 (1998) R212–R219.
[59] R. Gugler, M. Leschik, H.J. Dengler, Disposition of quercetin in man after single oral and intravenous doses, Eur. J. Clin. Pharmacol. 9 (1975) 229–234.
[60] N. Nishimoto, Interleukin-6 as a therapeutic target in candidate inflammatory diseases, Clin. Pharmacol. Ther. 87 (2010) 483–487.
[61] J.P. Spencer, Flavonoids: modulators of brain function? Br. J. Nutr. 99 E (Suppl 1) (2008) ES60–ES77.
[62] Y.J. Surh, Cancer chemoprevention with dietary phytochemicals, Nat. Rev. Cancer 3 (2003) 768–780.
[63] S.O. Rahaman, P.C. Harbor, O. Chernova, G.H. Barnett, M.A. Vogelbaum, S.J. Haque, Inhibition of constitutively active Stat3 suppresses proliferation and induces apoptosis in glioblastoma multiforme cells, Oncogene 21 (2002) 8404–8413.