Abstract. Purine analogue roscovitine, a cyclin-dependent kinase (CDK) inhibitor, has shown strong anti-proliferative and pro-apoptotic effects in solid and hematologic cancers such as non small-cell lung cancer and lymphomas. It targets CDK2, 7 and 9 preferentially, which are also overexpressed in glioblastoma. Therefore, the biological effects of roscovitine in glioblastoma cell lines were investigated. Glioblastoma A172 and G28 cell lines were incubated with serial concentrations of roscovitine for 24-120 h. Proliferation was measured using the xCELLigence Real-Time Cell Analyzer, an impedance-based cell viability system. Cell cycle distribution was assessed by flow cytometry and gene expression was quantified by quantitative RT-PCR and western blot analysis. Roscovitine exhibited a clear dose-dependent anti-proliferative and pro-apoptotic effect in the A172 cell line, while G28 cells showed a anti-proliferative effect only at 100 µM. The results of the flow cytometric (FACS) analysis revealed a dose-dependent increase of the G2/M and sub-G1 fractions in A172 cells, while G28 cells responded with an elevated sub-G1 fraction only at the highest concentration. Roscovitine led to a dose-dependent decrease of transcripts of p53, CDK 7 and cyclins A and E and an increase of >4-fold of p21 in A172 cells. In G28 cells, a dose-dependent induction of CDK2, p21 and cyclin D was observed between 10 and 50 µM roscovitine after 72 h, however, at the highest concentration of 100 µM, all investigated genes were downregulated. Roscovitine exerted clear dose-dependent anti-proliferative and pro-apoptotic effects in A172 cells and less distinct effects on G28 cells. In A172 cells, roscovitine led to G2/M arrest and induced apoptosis, an effect accompanied by induced p21 and a reduced expression of CDK2, 7 and 9 and cyclins A and E. These effects require further studies on a larger scale to confirm whether roscovitine can be used as a therapeutic agent against glioblastoma.

Introduction

Glioblastoma multiforme (GBM) is common and is an aggressive brain tumor in adults. Despite the introduction of radiation therapy and chemotherapy with temozolomide in addition to surgery, general survival has been increased from 12 to 14 months only (1,2). Specific genetic changes have been classified and related to characteristic molecular pathways involved in the control of tumor development. With an increasing understanding of the molecular behaviour of tumors, specifically of GBM, variations in pharmacogenomic profiles in combination with radiation therapy potentially improve the outcome of therapy in this lethal disease. Schiffer et al reported that in glioblastoma, the highest quantity of apoptosis was detected in the area with the highest mitotic rate suggesting that, during mitosis, some glioblastomas are exposed to cell death (3).

Uncommon regulation of cyclins, cyclin-dependent kinases (CDKs) and endogenous CDK inhibitors has been described in gliomas, demonstrating that CDK modulators may be important for treatment of GBM (4-9). Roscovitine, a CDK modulator is a potent and selective small molecule inhibitor of the cyclin-dependent kinases CDK2/cyclin B, CDK2/cyclin A, CDK2/cyclin E and CDK5, and leads to induction of p53 (10,11). Furthermore, roscovitine inhibits the growth of several human cancer cell lines including breast, ovarian, pancreatic, colon, renal, hepatocellular, pituitary carcinoma and neuroblastoma (12-18). Roscovitine has been found to inhibit different solid and hematologic tumor cell lines including acute lymphoblastic leukemia (ALL), which occurs frequently in children and is correlated with the central nervous system (CNS) (19,20). The drug inhibits the G1/S and G2/M transition in a concentration dependent manner.
Other studies reported an inhibition of the DNA synthesis in the cerebral cortex, an effect that appears to be independent from its ability to inhibit CDKs or the replication licensing factor (RLF) (10,11,21). The concentration and half-life of roscovitine are almost similar in plasma and in brain tissues. Roscovitine is metabolized in humans mainly by CYP3A4 and CYP2B6 enzymes, and its elimination is rapid via the bile within the first 24 h (22,23). Most chemotherapeutic agents do not cross the blood-brain barrier and do not reach the CNS in adequate concentrations to eliminate tumor cells, while roscovitine is highly distributed over the blood-brain barrier (24). Roscovitine is a potential inhibitor of CDK5, which has a significant function in the developing brain, such as neuronal migration (23). A functional p53 protein level was suggested as an efficient enhancer in roscovitine-induced apoptosis in cancer. Roscovitine-induced apoptosis was shown to be p53-dependent in MCF-7 cells, whereas roscovitine may induce apoptosis in B-CLL cells, irrespective of the functional status of the p53 pathway, and may be considered a therapeutic agent able to improve the outcome of B-CLL-resistant tumors. Therefore, the possible relationship between roscovitine and p53 regulation remains to be elucidated (25-27). Furthermore, roscovitine decreased production of the cell cycle inhibitor p21 and induced apoptosis. This effect was observed as the most efficient in cell lines expressing p53 protein with a full activity. The cells expressing partially and conditionally active p53wt mutants responded to roscovitine less efficiently. This observation suggests that patients with tumors exhibiting p53 can benefit from roscovitine therapy (28).

In the present study, we investigated the effects of roscovitine on proliferation, apoptosis and cell cycle regulation in glioblastoma cell cultures. Due to selective inhibition of CDKs by roscovitine, particularly CDK1, 2, 5, 7 and 9, which are involved in the cell cycle regulation, we assessed the interaction between roscovitine and CDKs. Furthermore, we investigated CDK binding partners including cyclin A, D and E and tumor-suppressor proteins p21 and p53. The results identified the influence of roscovitine in apoptosis in glioblastoma cell line as a promising therapeutic agent.

Materials and methods

Cell culture. The A172 and NCE-G28 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The two cell lines were routinely cultured in plastic flasks (75 cm²) or 6-well-plates in Dulbecco’s modified Eagle’s medium (DMEM; Biochrom GmbH, Berlin, Germany) supplemented with 10% fetal bovine serum (FBS; Biochrom) and 1 ppm antibiotics (penicillin and streptomycin; Biochrom). The cells were incubated at 37°C, under 95% humidity and 5% CO₂. At 70-80% confluence, the cells were passaged by being washed with PBS and trypsinized with trypsin/EDTA (both from Biochrom) for 3-7 min. PBS and trypsin/EDTA were preheated at 37°C in a water bath. The procedures were performed under sterile conditions in a laminar flow cabinet.

Drugs and treatments. Roscovitine was prepared as a 1M stock solution in dimethyl sulfoxide (DMSO; Sigma Aldrich, Hamburg, Germany). Stock solution was diluted in culture medium in order to obtain the final concentrations of 1, 10, 25, 50 and 100 µM for the cell treatment. Aliquots of the roscovitine stock and the final concentrations were stored until use at -20°C. The cells were treated with roscovitine 24 h after seeding to guarantee adherence. In the presence of the drug, the cells were incubated for 24-96 h. Controls were treated with complete medium as described above while omitting the drug.

Flow cytometry (FACS). Cell cycle analysis was performed using flow cytometry. Cells were seeded in triplicates in 6-well plates (3x10⁵/well) and allowed to attach for 24 h. Subsequently, the cells were treated with roscovitine at the final concentrations of 10, 25, 50 and 100 µM. After an additional incubation period of 24-96 h, the cells were washed with PBS (Biochrom) and collected by standard trypsinization. The collected medium, the PBS and the cell-trypsin suspension were centrifuged for 10 min at 1,000 rpm. Cell pellets were resuspended with propidium iodide (PI; Sigma Aldrich) at a ratio of 10⁵ cells/ml and incubated for 60 min on ice in the dark. Analysis was immediately performed. For the evaluation of the assays and the data, the BD FACSCalibur flow cytometer and the corresponding CellQuest™ Pro Software (BD Biosciences, San Jose, CA, USA) were used.

RNA isolation, cDNA synthesis and real-time-quantitative PCR (RT-qPCR). Cells were collected using flow cytometry and the corresponding CellQuest™ Pro Software (BD Biosciences, San Jose, CA, USA) was used.
(QT00057575), cyclin E (QT00063511), p21 (QT00062090), p53 (QT00060235) and GAPDH (QT01192646) (all from Qiagen). RT-qPCR was performed on a CFX96 Real-Time PCR system (Bio-Rad).

Protein isolation and western blot analysis. Cells were trypsinized as described before, centrifuged for 12 min at 4°C and 1,000 rpm, resuspended and washed in 1 ml PBS and pelleted at 1,000 rpm for 12 min. The pellet was resuspended in 80-200 µl Jie’s Buffer and allowed to dissolve on ice for 30 min with intermittent vortexing. After centrifugation at 13,000 rpm for 30 min the protein-enriched suspension was transferred into a new tube and stored at -80°C until further processing.

Protein quantification was performed using the Pierce BCA Protein Assay kit (Thermo Scientific) as per the manufacturer’s instructions. The readout was carried out on a SoftMax microplate reader using the Softmax Pro 3.11 software (Molecular Devices, Sunnyvale, USA). Gel electrophoresis was performed using a 30 µg protein solution. Antibodies were incubated using the Snap i.d. protein detection system (Millipore, Billerica, MA, USA) according to the manufacturer’s instructions. Chemiluminescence detection was performed with the Fusion-SL4 system and the Fusion-Capt software 15.15 by using the Pierce™ ECL Western Blotting Substrate (Thermo) and quantified using the Bio-1D package v.15.01 software (all from Vilber Lourmat, Eberhardzell, Germany). β-actin was used as the loading control. The antibodies used were: anti-Cdk2 (rabbit polyclonal, 1:300, cat no. ab6538) and anti-p21 (mouse monoclonal EA10, 1:100, ab16767; both from Abcam, Cambridge, UK), anti-β-actin (mouse monoclonal, clone AC-15, 1:3,000), and anti-mouse and anti-rabbit secondary antibodies (1:3,000, all from Sigma-Aldrich, St. Louis, USA).

Statistics. Statistic analysis and scientific graphing were performed using SPSS 20 (IBM software; Ehningen, Germany) and Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA). The flow cytometric results were analyzed using the ANOVA test and corrected using the Dunnett’s multiple comparison test for multiple comparisons. P<0.05 was considered statistically significant.

Results

Proliferation analysis. The effects of roscovitine on proliferation were examined in A172 and G28 glioma cell lines. Roscovitine induced a dose-dependent growth inhibition in A172 cells up to 50 µM and a distinct reduction of cell viability at 100 µM already after 24 h of treatment. IC₅₀ at 72 h was 67.55 µM. IC₅₀ at 72 h was decreased to 9.12 µM in the A172 cells and to 2.02 µM in G28 cells. Under
these conditions, there was an evident dose-dependent effect in G28 cells, in contrast to culturing with 10% FBS. Proliferation was similar under both conditions. The cells were monitored for up to 96 h to demonstrate continued proliferation even under nutrition-deficient conditions. The A172 cells reached a mean normalized cell index (CI) in the untreated controls of 4.4 after 96 h (vs. 3.6 when grown with 10% FBS). However, the G28 cells proliferated stronger with a mean CI after 96 h of 6.2 (vs. 2.5 when grown with 10% FBS). For comparable results with other studies on these cell lines, all subsequent experiments were performed using 10% FBS.

Cell cycle analysis by flow cytometry. To determine by which mechanism roscovitine inhibited cell proliferation, we analyzed cell cycle distribution by flow cytometry. After 72 h incubation, there was a clear dose-dependent increase of the pre-G1 cell fraction in the A172 cells from 2.5% in the controls, to 4.4, 3.9, 8.7 and 16.7% at 10, 25, 50 and 100 µM, respectively (Fig. 2A). At the same time-point, there was an increase of the G2/M fraction from 26.9 in the controls to 29.7, 37.8, 48.2 and 54.5% at these concentrations. The G1/S percentage was reduced from 70.6 in the controls to 28.8% in cells treated with 100 µM roscovitine. After 96 h, the percentage of apoptotic cells reached 54.2% at 50 µM roscovitine and 55.1% at 100 µM, respectively, vs. 3.6 in the untreated controls (Fig. 2B). The G1/S phase decreased from 80.8 in the controls to 72.9% at 100 µM roscovitine at 72 h. The G2/M cell fraction fluctuated at 61.2, 64.2 and 75.0% at 10, 25 and 50 µM, and only at 100 µM roscovitine was the level reduced to 34.9% (vs. 69.6 in the untreated controls).

Expression analysis of cell cycle regulator genes. A qPCR analysis for cell cycle regulators cyclins A, D and E, CDK2, CDK7, CDK9, p21 and p53 was performed in the two cell lines after 72 h incubation with roscovitine at 10, 25, 50 and 100 µM. Under incubation with roscovitine at 10, 25, 50 and 100 µM for 72 h, we measured dose- and time-dependent changes in all the genes investigated. In A172 cells, roscovitine induced the expression of CDK2 and cyclin D and suppressed p21, p53, CDK7, CDK9, cyclins A and E at 72 h. Under incubation with roscovitine at 10, 25, 50 and 100 µM for 72 h, we measured dose- and time-dependent changes in all the genes investigated. In A172 cells, roscovitine induced the expression of CDK2 and cyclin D and suppressed p21, p53, CDK7, CDK9, cyclins A and E at 72 h. At the growth-inhibiting concentrations of 10, 25 and 50 µM, CDK2 expression was increased 1.1-, 1.3- and 1.5-fold, respectively. At 100 µM, the concentration leading to cell death, decreased to 0.3-fold of the untreated controls. The expression of p21 was steadily induced over the dosing range, reaching a peak of 4.6-fold at
Figure 3. Relative gene expression in A172 and G28 cells treated with roscovitine at concentrations of 10-100 µM. Gene expression was determined by qPCR and normalized against GAPDH and untreated controls.

Figure 4. Western blot analysis of A172 and G28 cells treated with roscovitine. Panels show independent duplicate experiments of (A) CDK2 and (B) p21 expression in A172 and in G28 cells (C, CDK2; D, p21). Numbers show the densitometrically determined expression values after normalization to β-actin and the untreated controls.
100 µM roscovitine vs. the untreated controls. The expression of p53, CDK7 and 9 and cyclins A and E was gradually suppressed with increasing roscovitine concentrations and at 100 µM reached values of 0.3-, 0.02-, 0.04- and 0.01-fold of the untreated controls, respectively. Cyclin D was not detected using qPCR in these cells.

In the less roscovitine-sensitive G28 cells, the gene expression patterns followed the trend identified in A172 cells, reaching expression levels of 0.3 for p53, 0.5-fold for CDK7, 0.2-fold for CDK 9, 0.03-fold for cyclin A and 0.1-fold for cyclin E. CDK 2 also increased slightly and steadily at 10, 25 and 50 µM (1.3, 1.4 and 1.4-fold) and at 100 µM showed similar decreases as identified in A172 cells (0.3-fold). In contrast to the A172 cells, G28 cells expressed cyclin D, which was induced by the lower roscovitine concentrations of 10 and 25 µM (2.1- and 2.8-fold, respectively) and decreased at 50 and 100 µM to 1.0- and 0.5-fold, respectively. The expression of p21 was induced to 1.3-, 1.3- and 1.6-fold at 10, 25 and 50 µM and decreased to 0.2-fold at 100 µM. The clear dose-dependent effects observed in the proliferation and cell cycle analyses of the A172 cells were reflected in the gene expression and cell cycle studies, while the roscovitine effects of the G28 cells was less definite in all the analyses at 10% FBS.

Analysis of CDK2 and p21 expression by western blot analysis. To confirm whether the expression changes observed at the mRNA level as a consequence of roscovitine treatment translated to the protein level, we analyzed the protein expression of CDK2 and p21 by western blot analysis. In the A172 cells, roscovitine treatment induced CDK2 expression already after 24 h, with the strongest upregulation evident at 50 µM (2.44-fold). At the longer incubation period of 48 h, CDK2 was upregulated 3.50-fold at 50 and 100 µM but only 1.66-fold at 25 µM. The protein expression of p21 was strongly induced with increasing concentrations already after 24 h to values of 4.06-, 10.41- and 43.93-fold at 25, 50 and 100 µM, respectively. A less notable upregulation was observed after 48 h with values reaching 0.86-, 1.17- and 2.30-fold of untreated controls for the same concentration range.

The concentration of 50 µM appeared to be most efficient in upregulating CDK2 in G28 cells. After 24 h, CDK2 was upregulated at the protein level to 1.50-fold of the untreated controls and 25 and 100 µM concentrations only resulted in values of 1.38- and 0.85-fold, respectively. In these cells, 72 h of incubation resulted in a suppression of CDK2 protein to 0.65-, 0.39- and 0.67-fold at 25, 50 and 100 µM, respectively. Levels of p21 at 24 h were reduced at 25 and 100 µM in these cells (0.22- and 0.39-fold, respectively) or were not altered at 50 µM (1.01-fold). After the longer incubation of 72 h, higher concentrations of roscovitine led to a strong downregulation of p21 to 0.19-, 0.18- and 0.13-fold at 25, 50 and 100 µM. Of note, the two cell lines behaved in an opposite manner with respect to CDK2 and p21 expression, i.e., while A172 cells upregulated CDK2 and p21 when challenged with roscovitine, G28 cells suppressed the two proteins.

Discussion

GBM is the most common and most aggressive primary brain tumor in adults. Despite intensive therapeutic efforts, the prognosis remains poor (1). A successful multimodal therapy in this cancer needs to target multiple pathways, achieve sufficient concentration at the site of action within the brain and the treatment administered should have few side effects.

In the present study, we investigated the effect on the expression of CDK2, CDK7, CDK9 and cyclin A, D and E by using roscovitine in glioblastoma cells. We demonstrated that roscovitine induced cell cycle arrest in the G28 and A172 cell lines, which was paralleled by a decreased expression of CDK7, CDK9 and cyclin A, D and E. Furthermore, roscovitine induced apoptosis and decreased cell proliferation of the GBM cells.

Lim et al suggested that several modes of action can be postulated by the gene expression patterns observed after treatment with roscovitine. One of these modes of action leads to a reduced expression of CDK7, CDK 9 and cyclin D, the release inhibition of E2F and therefore suppression of E2F-dependent transcription, as well as suppression of the transcription by inhibiting RNA polymerase II-dependent transcription (29).

Previous findings have shown that cyclin E is often over-expressed in human tumors and the expression of the p21 and p27 inhibitors is often suppressed during tumor growth. This result demonstrates an involvement of CDK2 in human cancer (18). Other studies have shown that roscovitine induces cell cycle arrest by inhibiting CDK2 through competition for ATP-binding (10,30). Our results show distinct expression patterns in the two investigated cell lines, suggesting that different modes of action may cause the cell cycle effects observed, i.e., while the CDK2 expression was suppressed only at the highest concentrations, we observed a clearer and dose-dependent effect for p21, p53, CDK7 and cyclin A. Our data suggest that the observed effects of roscovitine on proliferation and cell survival depend on the inhibition of various kinases, as shown by Bach et al (31). On the other hand, a potent inhibition of the CDK2/cyclin E complex by roscovitine has already been shown in clinical trials (32). Therefore, roscovitine affects more pathways simultaneously and its specific inhibition of CDKs is not its only effect.

Roscovitine also inhibits CDK7, forming an enzyme complex with cyclin H. These data provide a direct connection between cell cycle regulation and transcription, CDK7 and cyclin H which form CAK, and a constituent of the basal transcription factor TFII F, which phosphorylates serine residues within the heptapeptide repeat of the carboxyterminal domain (CTD) of RNA polymerase II (33).

Furthermore, roscovitine inhibits cyclin H, cyclinD/CDK4 and cyclinD/CDK6 complexes by inhibiting CDK7 and reducing the cyclin D expression. This is due to the fact that CDK activation requires cyclin binding for conformational changes in the tertiary structure as well as phosphorylation at a conserved threonine residue. Cyclin binding leads to conformational changes in the tertiary structure of the CDKs, including the ATP-binding side, which allows subsequent phosphorylation that is required for complete CDK activation. Phosphorylation is catalyzed by the CAK (34,35). CAK comprises a CDK7 complex with cyclin H and Mat1 (33). In quiescent cells and at the beginning of the G1 phase, E2F is controlled by pRB (35,36). As soon as pRB is phosphorylated by cyclinD/CDK4 and cyclinD/CDK6 and subsequently by cyclinE/CDK2, E2F is released and transcription initi-
ated (35,37). Collectively, CDK2, CDK4, CDK6 and cyclin D are necessary for transcription. Most probably, roscovitine inhibits the activation of cyclinD/CDK4 and cyclinD/CDK6 by suppressing the expression of CDK7 and cyclin D, leading to inhibition of the transcription. Thus, E2F-dependent transcription of cycle relevant proteins, including cyclin A and E, is suppressed, leading to cell cycle arrest at different stages. RNA-polymerase II performs the actual transcription. For activation it requires phosphorylation of its C-terminal domain (CTD). CDK7/cyclinH/Mat1 CAK, which are components of the TFIIH complex, and CDK9/cyclinT (pTEFb) phosphorylate the CTD of RNA polymerase II (38). Thus, roscovitine may also inhibit transcription by impairing RNA-polymerase II activation.

Our results are not consistent with those of other studies which showed an increased mRNA expression of cyclin A, B, D (32,39) and CDK7 and 9 (26,39) in various types of cancer, but not glioma cells.

Similar to our gene expression results in the glioblastoma cell lines, Whittaker et al showed reduced cyclin A and D expression at the protein level by western blot analysis in human colon cancer cells (32). This decrease in phosphorylation may contribute to or even cause the decreased cyclin expression. This increased phosphorylation may most likely be due to an inhibition of CDK7 and 9 (32,39). Similar mechanisms likely caused the observed effects in the present study, although functional analyses to confirm these results were not performed.

Previous findings showed that roscovitine induced cell cycle arrest and concomitantly apoptosis in tumor cells of breast and colon cancer (14,35) mostly due to mitochondrial-mediated apoptosis activation of caspase-3, -8 (40) and -9 (34). Our results are in agreement with observations by Węsierska-Gądek et al (26,30), showing that roscovitine induces G2/M-phase cell cycle arrest in tumor cells. In our study we observed progressive G2/M arrest accompanied by decreasing G1/S fraction in A172 and G28 cell lines. The A172 cells showed a time- and concentration-dependent G2/M arrest in almost all the samples, whereas G28 cells showed G2/M arrest after treatment with highest concentrations only. As the G2/M phase is known as the most radio-sensitive phase in the cell cycle (41), roscovitine may be useful in combination with radiotherapy, which is also part of the current therapeutic standard for glioblastoma.

McCue et al (22) identified that the major effect of roscovitine is not by cell cycle arrest in a specific phase but rather an induction of apoptosis in different cell cycle phases. However, findings by those authors cannot be supported with the data of the present study.

Regarding our FACS results, we identified a concentration- and time-dependent induction of apoptosis with roscovitine treatment, accompanied by a decreased CDK7 and 9 expression. Previous results have shown that roscovitine induces apoptosis by inhibiting RNA polymerase II-dependent transcription of the anti-apoptotic Bcl-2-family member Mcl-1 (myeloid cell leukemia 1) (38,40). Those studies showed a decreased CDK7 and 9 expression due to roscovitine treatment with a consecutive decrease of RNA polymerase II phosphorylation leading to its depression. Using qPCR we determined a decreased expression of CDK7 and 9. Even in this tumor entity, roscovitine was capable of inducing apoptosis by disabling the RNA polymerase II-dependent transcription of Mcl-1 due to a lack of CDK7 and 9 expression.

Previous studies reported a p53 increase in glioblastoma cells following roscovitine treatment (16,26). There are p53-dependent and -independent pathways described in the literature (10,16,18,26) with respect to apoptosis. However, there is a greater potency described against p53 wild-type cells than against mutant cells (10,16,18,26). In the present study, the expression response of p53 under roscovitine incubation was different in the two cell lines. This is explained by the fact that one of the most important differences between the two cell lines is the p53 status. A172 cells bear wild-type p53, while a mutated p53 is present in the G28 gliosarcoma cell line (42,43). It is likely that the stronger roscovitine influence identified in the A172 cells is likely due to the wild-type p53.

Previous findings have shown that roscovitine decreased the expression of p21 and p21 cleavage was involved in roscovitine induced apoptosis. Even in earlier studies on roscovitine it was presumed that a high expression of p21 inhibited the induction of apoptosis (28,44-46). That finding is similar to our results for the G28 cells. The inhibition of p21 was time- and concentration-dependent. The investigation of A172 showed similar results until 48 h. The expression of p21 after application of 100 µM of roscovitine after 72 h was higher. This result can be interpreted as a regulatory mechanism to escape apoptosis.

In conclusion, our pilot study demonstrates an anti-proliferative and pro-apoptotic effect of roscovitine on two human glioblastoma cell lines in vitro, accompanied by distinct changes in gene expression of CDKs and cyclins, along with p53 and p21. Given the good oral bioavailability of roscovitine and its effect on gene expression of CDKs and cyclins, along with p53 and p21, this drug should be investigated in depth in further pre-clinical and clinical studies as it shows to be a promising agent against glioblastoma alone or in combination therapy (24,47).

References