RESEARCH ARTICLE

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Dual Role of Colchicine in Glioblastoma: miR-22-3p Dependent Regulation of Wound Healing and Proliferation

Glioblastoma Hücrelerinde Kolçisinin Çift Yönlü Etkisi: miR-22-3p Aracılı Yara İyileşmesi ve Hücre Proliferasyonunun Düzenlenmesi

ABSTRACT

Objective

The downregulation of miR-22-3p in glioblastoma (GBM) tissues and cells has been shown to play a critical role in gliomagenesis and patient prognosis. However, the effects of colchicine on GBM cellular functions via miR-22-3p mediated mechanisms remain unexplored. This study aims to investigate the effects of colchicine on GBM cell behavior through miR-22-3p-mediated mechanisms.

Methods

miR-22-3p expression levels were quantified in U87 GBM cells treated with 1, 10, and 100 ng/ml colchicine using qRT-PCR. Cell viability and drug resistance were evaluated using the CCK-8 assay across four groups: temozolomide (TMZ) treated or untreated (i) U87 cells (control), (ii) U87 cells treated with 10 ng/ml colchicine, (iii) U87 cells transfected with a miR-22-3p inhibitor, and (iv) U87 cells transfected with a miR-22-3p inhibitor and 10 ng/ml colchicine. Colony formation was analyzed, migration was assessed using a transwell assay, and wound healing capacity was evaluated via scratch assay, all in U87 cells treated with 10 ng/ml colchicine.

Results

Colchicine at 10 ng/ml significantly upregulated miR-22-3p expression. Inhibition of miR-22-3p reversed the colchicine-induced reduction in cell viability and colony formation. Neither colchicine nor miR-22-3p affected the resistance of U87 cells to TMZ. Colchicine reduced U87 cell migration independently of miR-22-3p, while its effect on wound healing was dependent on miR-22-3p expression.

Conclusion

This study demonstrates that colchicine modulates miR-22-3p expression and influences key GBM cell functions. These findings provide a preliminary evidence supporting the potential of colchicine as a therapeutic agent in GBM treatment.

Key Words

Glioblastoma, Colchicine, miR-22-3p, Transfection, Proliferation, Migration, Wound healing

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ÖZ

Amaç

Glioblastoma (GBM) doku ve hücrelerinde miR-22-3p'nin regülasyonu, gliomagenez ve hasta prognozunda kritik bir rol oynamaktadır. Ancak kolçisinin, GBM hücresel fonksiyonları üzerindeki miR-22-3p aracılı mekanizmalar yoluyla olan etkileri henüz araştırılmamıştır. Bu çalışmanın amacı, kolçisinin GBM hücre davranışları üzerindeki etkilerini miR-22-3p aracılı mekanizmalar üzerinden incelemektir.

Yöntemler

U87 GBM hücrelerinde 1, 10 ve 100 ng/ml kolçisin uygulaması sonrasında miR-22-3p ekspresyon düzeyleri qRT-PCR ile ölçülmüştür. Hücre canlılığı ve ilaç direnci, CCK-8 testi kullanılarak dört grup üzerinden değerlendirilmiştir: temozolomid (TMZ) uygulanan ya da uygulanmayan (i) kontrol grubu U87 hücreleri, (ii) 10 ng/ml kolçisinle muamele edilen U87 hücreleri, (iii) miR-22-3p inhibitörü ile transfekte edilen U87 hücreleri ve (iv) hem miR-22-3p inhibitörü ile transfekte edilen hem de 10 ng/ml kolçisinle muamele edilen U87 hücreleri. Koloni oluşumu, koloni oluşum testi ile; migrasyon, transwell testi ile; yara iyileşmesi kapasitesi ise çizik (scratch) testi ile, 10 ng/ml kolçisin uygulanan U87 hücrelerinde analiz edilmiştir.

Bulgular

10 ng/ml kolçisin, U87 hücrelerinde miR-22-3p ekspresyonunu anlamlı düzeyde artırmıştır. miR-22-3p inhibisyonu, kolçisinin hücre canlılığı ve koloni oluşumu üzerindeki baskılayıcı etkisini tersine çevirmiştir. Kolçisin veya miR-22-3p, U87 hücrelerinin TMZ'ye olan ilaç direncini etkilememiştir. Kolçisin, hücre migrasyonunu miR-22-3p'den bağımsız olarak azaltırken, yara iyileşmesi üzerindeki etkisi miR-22-3p ekspresyonuna bağımlı olarak gözlenmiştir.

Sonuç

Bu çalışma, kolçisinin miR-22-3p ekspresyonunu düzenlediğini ve GBM hücrelerinin temel fonksiyonlarını etkilediğini göstermektedir. Bulgular, kolçisinin glioblastoma tedavisinde potansiyel bir terapötik ajan olarak değerlendirilmesine yönelik ön kanıtlar sunmaktadır.

Anahtar Kelimeler

Glioblastoma, Kolçisin, MiR-22-3p, Transfeksiyon, Proliferasyon, Migrasyon, Yara iyileşmesi

INTRODUCTION

Glioblastoma multiforme (GBM), the most aggressive subtype of glioma in adults, is highly malignant and prone to recurrence, accounting for approximately 45% of all malignant central nervous system tumors and 55% of gliomas (1). According to the World Health Organization (WHO) classification, GBM is categorized as a grade IV glioma and is associated with a poor prognosis, with a 5-year overall survival rate of less than 6% (1, 2). Despite the use of multimodal treatment approaches -including surgery, adjuvant radiotherapy, temozolomide (TMZ)-based chemotherapy, and targeted therapies such as rituximab the overall survival rate remains unsatisfactory, with a median survival time of only 14 months from initial diagnosis (2). Therefore, the development of novel targeted therapies, the optimization of combination treatment strategies, and a deeper understanding of the mechanisms underlying GBM malignancy are essential to improving diagnostic and therapeutic outcomes (3, 4).

Microtubules play a crucial role in cell mitosis, making them key targets for cancer therapy (5). Microtubule-targeting agents (MTAs) exert antitumor effects by disrupting microtubule dynamics, thereby interfering with mitotic spindle formation, arresting the cell cycle at the metaphase and anaphase, and ultimately inducing tumor cell apoptosis (5, 6). Several MTAs, including paclitaxel, vinblastine, vincristine and colchicine, have either been approved for clinical use or are currently under investigation (6). Colchicine, an alkaloid derived from the Autumn Crocus plant, exhibits greater selectivity for tumor cells compared to normal cells, thus reducing toxicity to healthy tissues and improving therapeutic efficacy (7). Additionally, MTAs that target the colchicine-binding site have demonstrated the ability to overcome acquired drug-resistance (8). Colchicine-binding site inhibitors (CBSIs) have also been shown to inhibit tumor angiogenesis and vascular disruption (9, 10). These mechanisms position colchicine as a promising anticancer agent capable of suppressing cancer cell migration, metastasis, and angiogenesis (7). Although the precise antitumor mechanisms of colchicine remain incompletely understood, growing evidence suggests that it may alter microRNA (miRNA) expression profiles (11-15). Among these, miR-22-3p has been found to be significantly downregulated in human GBM tissues and cell lines (16-20). Functionally, miR-22-3p has been reported to suppress tumor cell viability, migration, and invasion while promoting apoptosis (16-18, 21). Moreover, elevated expression of miR-22-3p enhances the sensitivity of glioma cells to cisplatin, highlighting its potential role in improving therapeutic outcomes (20, 21). However, whether colchicine exerts its antitumor effects in GBM through the modulation of miR-22-3p remains to be elucidated.

In this study, we hypothesized that colchicine may exert part of its antitumor effects in GBM by modulating the expression of miR-22-3p, thereby inhibiting cancer cell viability and migration, and potentially enhancing chemosensitivity. The first objective was to assess the expression of miR-22-3p in U87 cells treated with varying concentrations of colchicine. The second objective was to evaluate the effects of three different colchicine doses-administrated alone or in combination with a miR-22-3p inhibitor-on cell viability, colony formation, TMZ resistance, and to determine the IC_{50} value. The third objective was to investigate the effects of colchicine and/or miR-22-3p inhibition, at cytotoxic concentrations, on U87 cell migration and wound healing capacity.

MATERIALS and METHODS

Study design

We conducted a prospective, randomized, controlled in vitro study. The independent variables were experimental groups, which included U87 cells treated or untreated with temozolomide (TMZ) and/or colchicine, as well as U87 cells transfected with either has-miR-22-3p antagomir (anta-miR-22 group) or a miRNA mimic negative control (NC group). The dependent variables included quantitative measurements of miRNA expression, cellular metabolic activity, colony formation, migration, and wound healing. RT-PCR, cell viability, colony formation, migration and wound healing assays were repeated independently two times, with each condition plated in at least triplicate wells. A schematic representation of the experimental setup is presented in Fig. 1a.

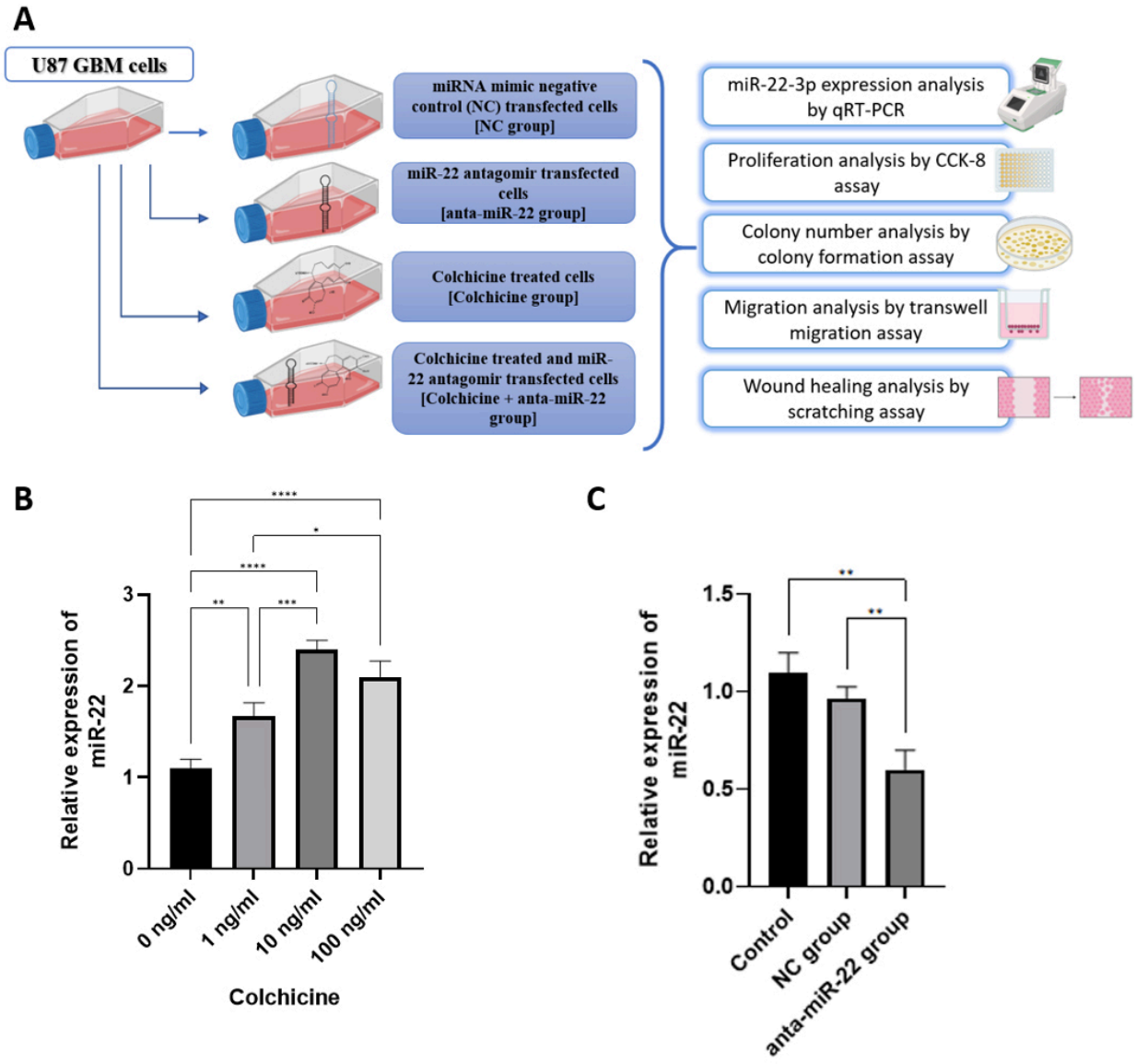

Fig. 1 qRT-PCR analysis revealing that colchicine increases miR-22-3p expression in a dose-dependent manner in U87 cells. (A) Schematic overview of the experimental design. (B) miR-22-3p expression was significantly upregulated in U87 cells treated with 1, 10, and 100 ng/ml colchicine compared to untreated control cells; (C) miR-22-3p expression significantly downregulated in U87 cells transfected with miR-22 antagomir (anta-miR-22 group) compared to nontransfected (control) and negative control miRNA mimic transfected (NC group) cells (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001) (n=3).

Cell culture

The human glioblastoma cell line U87 MG (U87, #HTB-14) was obtained from the American Type Culture Collection (ATCC, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, #41965039, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; PAN Biotech, #P30-193306, Germany) and 1% (v/v) antibiotic solution containing 10,000 U/mL penicillin and 10,000 µg/mL streptomycin (Biochrom, Germany). Cells were maintained at 37°C in a humidified incubator with 5% CO_2 (22).

RNA isolation and quantitative real-time PCR

Total RNA was isolated using the Ecopure Total RNA kit (Ecotech biotech, #E2075, Turkey) according to the manufacturer's protocol. For miRNA expression analysis, RNA was reverse-transcribed into complementary DNA (cDNA) using the miRNA All-In-One cDNA Synthesis Kit (ABM, #G898, Canada). Quantitative real-time PCR (qRT-PCR) was performed using A.B.T. 2X qPCR SYBR-Green MasterMix (#Q03-02-05, Turkey) on a CFX96 Real-Time PCR Detection System (Bio-Rad, USA). Relative expression levels and transfection efficiency were calculated using the 2-ΔΔCT method (23). U6 small nuclear RNA (snRNA; MS00033740) was used as an internal control. The primer sequences were as follows: U6, forward: 5′-GCTTCGGCAGCACATATACTAAAAT-3′, reverse: 5′-CGCTTCACGAATTTGCGTGTCAT-3′; miR-22-3p, forward: 5`-GTTCTTCAGTGGCAAGC-3`, reverse: 5`-GAACATGTCTGCGTATCTC-3`.

Transfection

U87 cells were seeded at a density of 2 × 10^5 cells/well. Transfection was performed using Lipofectamin Transfection Reagent (Invitrogen, #18324012, USA) with either hsa-miR-22 antagomir (anta-miR-22; ABM, #MNH01581) or miRNA mimic negative control (NC; ABM, #MCH00000), following the manufacturer's protocol (24).

Cell viability assay

Cell viability was evaluated using the Cell Counting Kit-8 (CCK8, Abbkine, #KTA1020, China) according to the manufacturer's protocol. miR-22-3p transfected U87 cells, treated with or without 212.3 µM TMZ (Kocak Farma, Temomid, Turkey), were seeded in 96-well plates at a density of 1 × c cells per well (25). After 48 h of incubation, 10 µL of CCK-8 solution was added to each well, followed by an additional 4h incubation period. Absorbance was measured at 450 nm using a microplate reader (Thermo Scientific, Multiscan GO, USA) (26). On day 3, the half-maximal inhibitory concentration (IC_{50}) and coefficient of determination (R^2) for colchicine were calculated using GraphPad Prism Version 10.1.2 (La Jolla, CA, USA).

Colony formation assay

U87 cells were seeded into 24-well plates at a density of 1 × 10^2 cells per well in 0.8% agar (Sigma, #A7921) prepared in growth medium, layered over a 1% agar base. The plates were incubated for 14 days to allow for visible colony formation. Colonies were then stained with 0.2% crystal violet, visualized under a microscope, and photographed. Colony numbers were quantified using the Axiocam imaging system and Zen 3.3 software program (27).

Transwell migration assay

Migration assays were performed using 24-well transwell inserts with 0.5 µm pore size (Corning, #3422, USA). Briefly, 5 × 10^4 cells were seeded into the upper chamber in 100 µL of serum-free medium, while 600 µL of complete growth medium was added to the lower chamber. After 48 h of incubation, non-migrated cells on the upper surface of the membrane were removed by washing with cold PBS. The migrated cells were then fixed with 3.7% paraformaldehyde (PFA) and stained with 0.2% crystal violet (Merck, #109218, Germany) for 30 min at room temperature. Stained cells were visualized under a microscope (Zeiss, Primovert, Germany), and images were captured using the Axiocam imaging system and the Zen 3.3 software (Zeiss). Quantification was performed using the ImageJ software (NIH, USA) by analyzing five randomly selected fields from each of the three membranes (23, 28).

Wound healing

U87 cells were seeded into 24-well plates at a density 1 × 10^5 cells/well. Once a confluent monolayer was achieved, a linear scratch was created using a 200-µL micropipette tip, and the wells were washed with PBS to remove any detached cells. The cells were then incubated under standard culture conditions. Wound areas were visualized at 0 and 48 h using a microscope at 10× magnification, and photographs were captured. The wound area was quantified using the Zen 3.3 software. Wound closure was calculated by comparing the wound area at the beginning and end of the assay (16).

Statistical analysis

Data are presented as the means +/– SD and analyzed using GraphPad Prism Software. One-way and two-way ANOVA with Tukey's multiple comparisons test was used for comparisons between multiple groups. A p-value of < 0.05 was considered statistically significant.

RESULTS

Colchicine upregulates miR-22-3p expression in a dose-dependent manner in glioblastoma cells

We observed that miR-22-3p expression was significantly upregulated in U87 cells treated with 1 ng/ml ($p<0.01$), 10 ng/ml ($p<0.0001$), and 100 ng/ml ($p<0.0001$) colchicine compared to untreated control cells (Fig. 1b). Moreover, miR-22-3p expression in the 1 ng/ml colchicine group was significantly lower than in the 10 ng/ml ($p<0.001$)

and 100 ng/ml ($p<0.05$) colchicine treated cells (Fig. 1b). The highest expression level of miR-22-3p was detected in U87 cells treated with 10 ng/ml colchicine (Fig. 1b). In addition, transfection with miR-22-3p inhibitor significantly downregulated miR-22-3p expression in U87 cells compared to both untransfected control cells (control) ($p < 0.01$) and NC miRNA mimic-transfected cells (NC group) ($p < 0.01$) (Fig. 1c).

miR-22 inhibition reverses the colchicine-induced reduction in cell viability in glioblastoma

TMZ treatment (TMZ(+)) significantly reduced U87 cell viability across all experimental groups compared to untreated cells (TMZ(-)) ($p < 0.05$) (Fig. 2a, b, c). Notably, both TMZ(+) and TMZ(-) miR-22 antagomir transfected cells (anta-miR-22 group) exhibited higher viability than TMZ(+) and TMZ(-) negative control miRNA mimic transfected cells (NC group), colchicine-treated (colchicine group) and colchicine-treated anta-miR-22 group (colchicine + anta-miR-22 group) at all colchicine concentrations ($p < 0.05$) (Fig. 2a, b, c). Furthermore, TMZ(-) colchicine + anta-miR-22 group showed higher viability than TMZ(-) colchicine group at all concentrations ($p < 0.05$) (Fig. 2a, b, c). TMZ(+) colchicine + anta-miR-22 group showed higher viability than TMZ(+) 10 and 100 ng/ml colchicine group ($p < 0.05$) (Fig. 2b, c). We also found that TMZ(+) cells treated with 10 and 100 ng/ml colchicine had lower viability compared to TMZ(+) NC group and TMZ(+) anta-miR-22 group cell viability, respectively ($p < 0.05$) (Fig. 2b, c). Additionally, both TMZ(-) and TMZ (+) NC group displayed higher viability than those treated with 10 and 100 ng/ml colchicine group ($p < 0.05$) (Fig. 2b, c). The IC_{50} value for colchicine in U87 cells was calculated as 5.88 nM on day 3 (Fig. 2d).

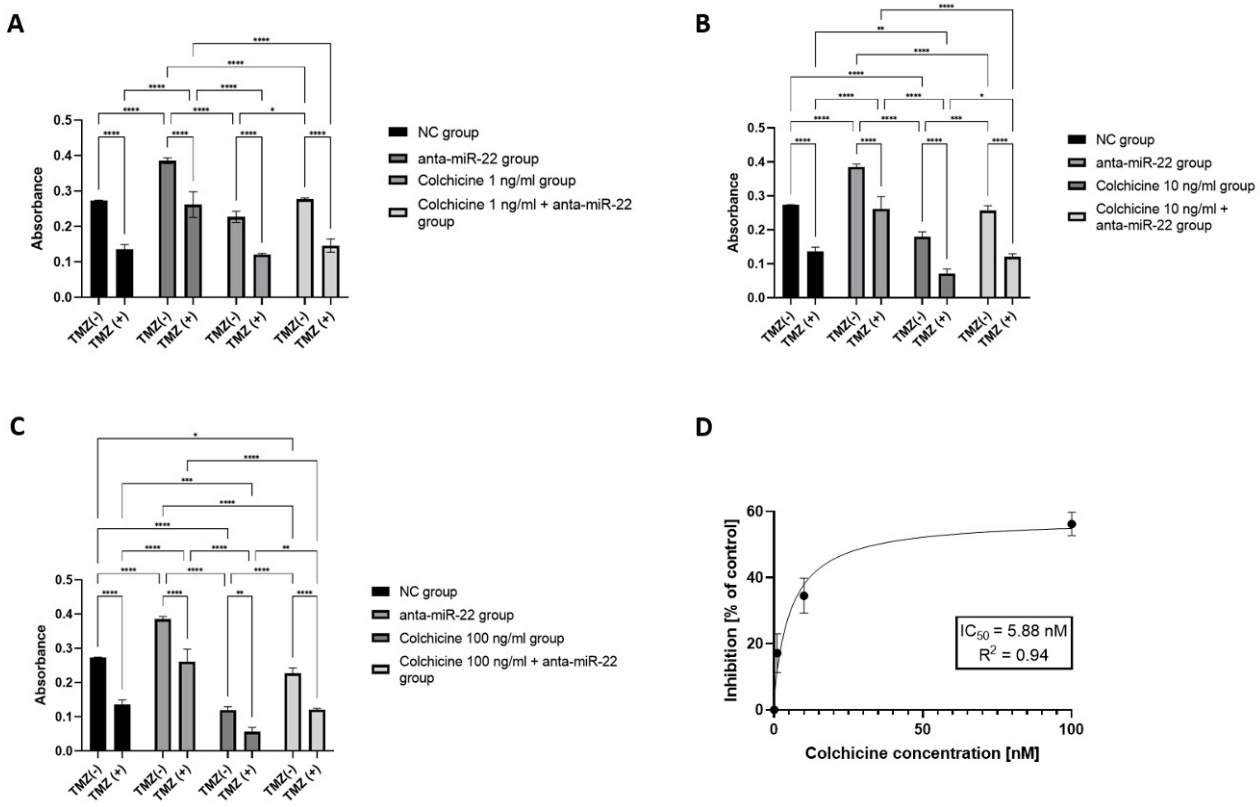

Fig. 2 Inhibition of miR-22 reverses the colchicine-induced reduction in cell viability in glioblastoma. Viability analysis of U87 cells treated with temozolomide (TMZ) and (A) 1, (B) 10, and (C) 100 ng/ml colchicine, following transfection with either negative control miRNA mimic (NC group) or miR-22 antagomir (anta-miR-22 group) on day 3. (D) The half-maximal inhibitory concentration (IC_{50}) (nM) and coefficient of determination (R^2) values for colchicine in U87 cells (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001) (n=3).

miR-22 inhibition reverses the colchicine-induced reduction in colony formation in glioblastoma

Microscopic analysis revealed that colchicine treatment reduced, while miR-22 inhibition enhanced, the colony formation capacity of U87 cells (Fig. 3a). Transfection with miR-22 antagomir (anta-miR-22 group) led to a significant increase in colony numbers compared to the NC group ($p < 0.01$), colchicine group ($p < 0.0001$), and colchicine+anta-miR-22 group ($p < 0.001$) (Fig. 3b). In addition, the number of colonies in the colchicine group was significantly lower than in both the NC group ($p < 0.001$) and the colchicine+anta-miR-22 group ($p < 0.01$) (Fig. 3b).

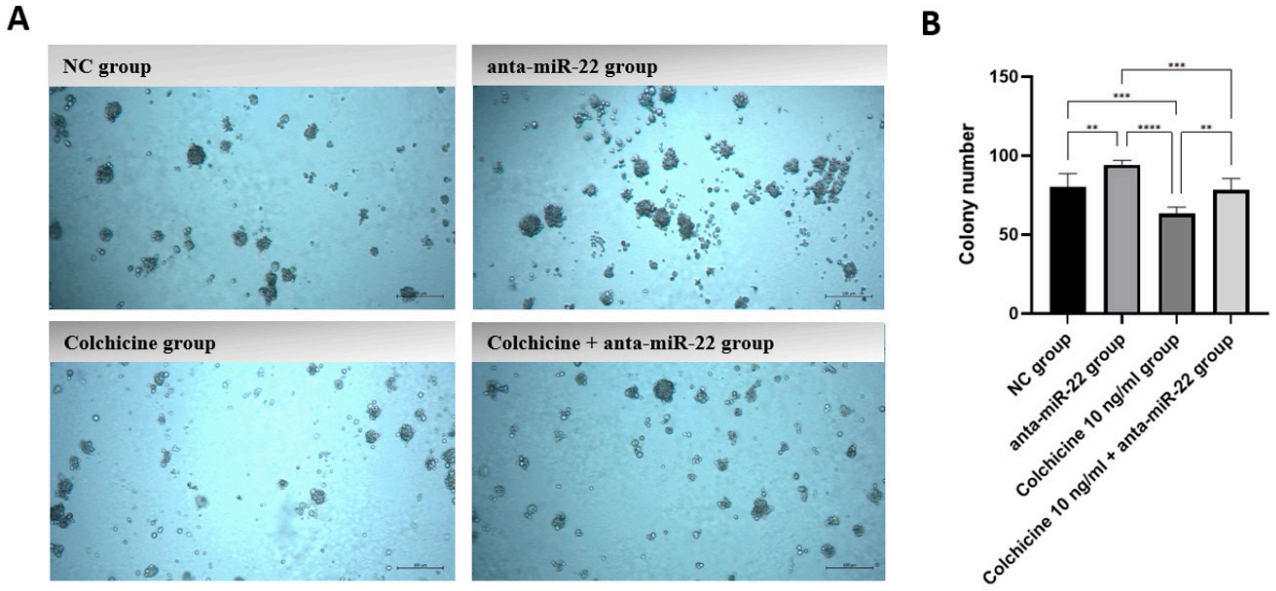

Fig. 3 Inhibition of miR-22 reverses the colchicine-induced reduction in colony formation in glioblastoma. (A) Morphologic analysis of colonies (10×) and (B) quantification of colony numbers in U87 cells transfected with negative control miRNA mimic (NC group) or miR-22 antagomir (anta-miR-22 group) and treated with or without colchicine (10 ng/mL) on day 14 (**p<0.01, ***p<0.001, ****p<0.0001) (n=3) (scale bar, 100 µm).

Colchicine reduces glioblastoma cell migration independently of miR-22

Microscopic analysis demonstrated that inhibition of miR-22 enhanced, whereas colchicine treatment suppressed, the migration capacity of U87 cells (Fig. 4a). Transfection with miR-22 antagomir significantly increased the number of migrated cells compared to both the NC group ($p < 0.0001$) and colchicine group ($p < 0.0001$) (Fig. 4b). In contrast, colchicine treatment significantly reduced the number of migrated cells compared to NC group ($p < 0.01$), anta-miR-22 group ($p < 0.0001$), and colchicine+anta-miR-22 group ($p < 0.0001$) (Fig. 4b). The migrated cell number was higher in colchicine+anta-miR-22 group compared to NC group ($p < 0.01$) (Fig. 4b).

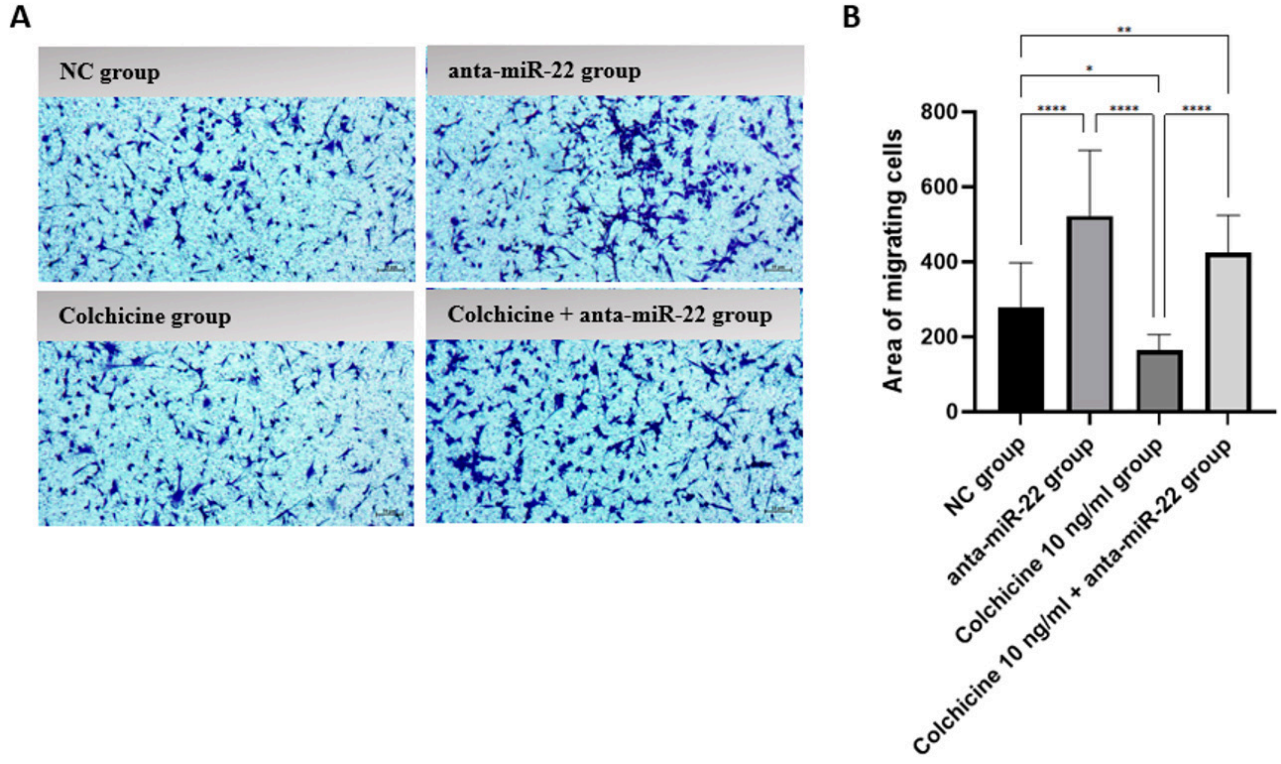

Fig. 4 Colchicine reduces glioblastoma cell migration independently of miR-22. (A) Morphologic analysis of the migrated cells and (B) quantification of migrated cell areas in U87 cells transfected with negative control miRNA mimic (NC group) or miR-22 antagomir (anta-miR-22 group) and treated with or without colchicine (10 ng/mL) on day 2 (10×) (*p<0.05, **p<0.01, ****p<0.0001) (n=3) (scale bar, 10 µm).

Colchicine reduces glioblastoma cell wound healing ability independently of miR-22

Microscopic analysis showed that inhibition of miR-22 increased, while colchicine treatment decreased, the wound closure ability in U87 cells compared to the NC group (Fig. 5a). miR-22 antagomir inhibitor (anta-miR-22 group) transfection led to a significant increase in the wound closure ability relative to NC group ($p < 0.05$), colchicine group ($p < 0.01$), and colchicine+anta-mir-22 group ($p < 0.05$) (Fig. 5b). There was no significant difference in wound area measurements between colchicine group and colchicine+anta-mir-22 group ($p > 0.05$) (Fig. 5b).

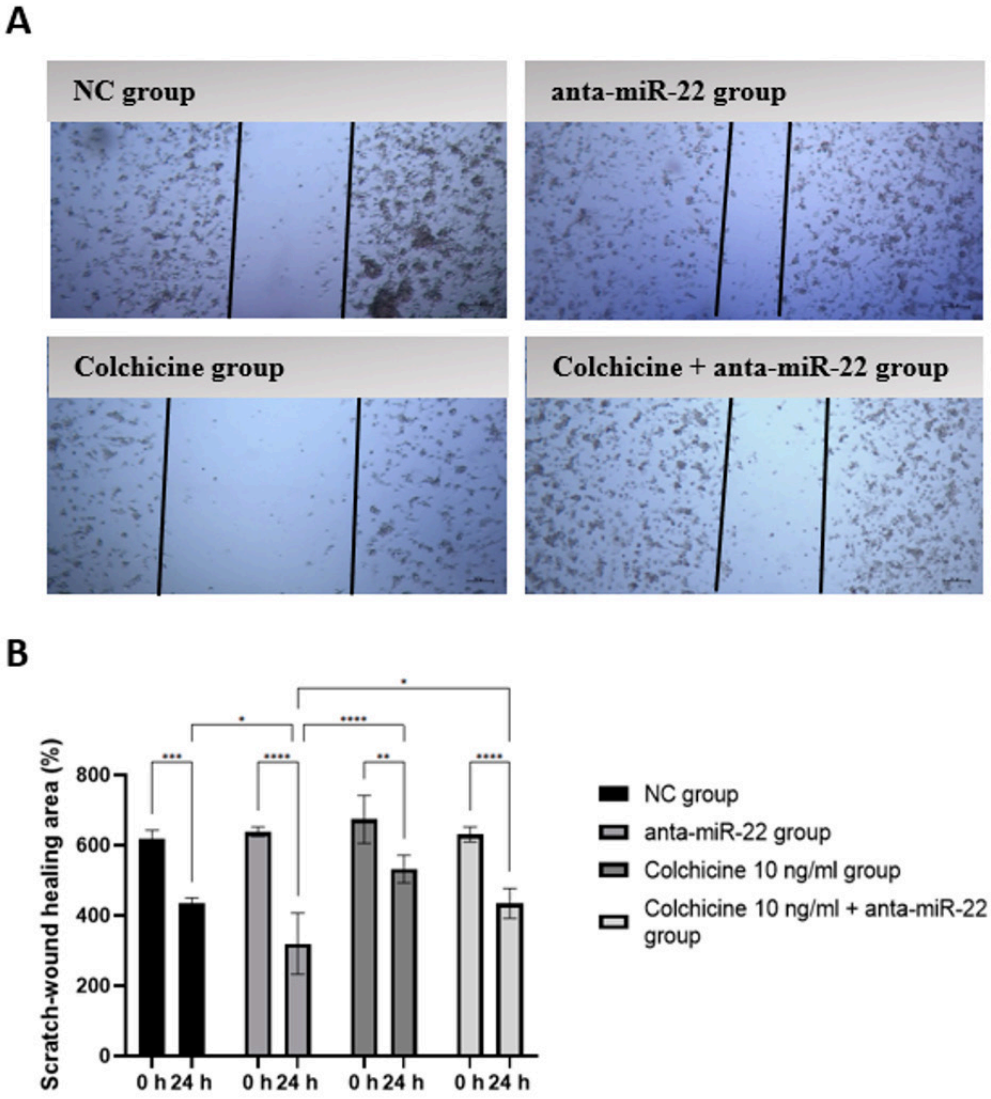

Fig. 5 Colchicine reduces glioblastoma wound healing independently of miR-22. (A) Morphologic analysis of the wound closure and (B) quantification of wound closure in U87 cells transfected with negative control miRNA mimic (NC group) or miR-22 antagomir (anta-miR-22 group) and treated with or without colchicine (10 ng/mL) on day 2 (10×) (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001) (n=3) (scale bar, 50 µm).

DISCUSSION

We found that miR-22-3p expression was upregulated in U87 cells treated with 1, 10, and 100 ng/ml colchicine compared to untreated controls. Although there is no direct evidence linking colchicine to miR-22-3p regulation, previous studies have demonstrated that colchicine can modulate the expression of various microRNAs. In a study conducted on patients with acute coronary syndrome, the levels of twelve microRNAs detected in the peripheral blood (PB) plasma were reduced following colchicine treatment, although no specific data were reported for miR-22-3p (11). Similarly, an increase in miR-21 was observed in the PB plasma of the Behçet's Disease mouse model, while qRT-PCR analysis revealed that miR-21 expression decreased after colchicine administration (2 µg/mouse) (12). Another study analyzing PB plasma from patients with Familial Mediterranean Fever (FMF) showed that five microRNAs were significantly upregulated, while five others were downregulated in response to colchicine treatment (13). Moreover, three microRNAs exhibited significantly altered expression in colchicine-resistant FMF patient samples (14). In colchicine-treated patients with acute gouty arthritis (AGA), qRT-PCR analysis revealed a significant upregulation of miR-223-3p expression (15). Taken together, these findings suggest that colchicine has the potential to modulate microRNA expression under various pathological conditions. While no prior studies have specifically addressed the regulation of miR-22-3p by colchicine, our results provide the first evidence that colchicine treatment may induce upregulation of miR-22-3p in glioblastoma cells. This novel finding raises the possibility that miR-22-3p may contribute to the anti-tumoral mechanisms of colchicine, highlighting the need for further investigation into its functional role in glioblastoma biology.

We found that miR-22 inhibition reversed the colchicine-induced reduction in cell viability in U87 cells. Although direct evidence on colchicine itself in GBM models is limited, studies involving colchicine analogues or derivatives have reported promising anti-tumor activity. In C6 rat GBM cells treated with 10, 20, 40 and 250 µM colchicine, an 80% and 40% statistically significant decrease in viability detected at 24 h and 60 min, respectively, using MTT viability analysis (29-31). In our study, we found a significant decrease of 35% and 48% in U87 cell viability following treatment with 0.025 and 0.25 µM colchicine, respectively, on day 3. These differences may be attributed to variation in treatment duration (24 h or 60 min vs. 3 days). Similarly, treatment with the colchicine derivative AD1 caused a 50% decrease in viability in U87 and U373 human GBM cells after 24 h, measured by trypan blue exclusion assay (32). This aligns with our findings, which showed a significant decrease of 35% and 48% in U87 cell viability with 25 and 250 nM colchicine, respectively, compared to the control on day 3. In other cancer models, a 10% decrease in viability was reported in A549 human non-small lung carcinoma epithelial cells treated with 2.5 nM colchicine for 72 h, as measured by MTT (33). Likewise, a 15% reduction in 4T1 murine breast cancer cell viability was detected after 1 nM concentration treatment for 24 h using the CCK-8 assay (34). These results are in line with our observation, where we observed a 15% reduction in U87 cell viability with 2.5 nM colchicine. Furthermore, in human hypopharyngeal cancer cell lines treated with 10 and 100 nM colchicine, cell viability was reduced by 55% and 68% in FaDu cells, and by 10% and 47% in SNU1041 cells on day 3, according to XTT analyses (35). In human osteosarcoma cell lines Saos-2 and U2OS treated with 50 nM colchicine, 55% and 35% inhibition in cell viability was observed, respectively, on day 2 as determined by CCK-8 assay (36). These results are consistent with our findings, where colchicine at 25 and 250 nM caused 35% and 48% reductions in U87 cell viability on day 3, respectively. Although MTAs that bind to the colchicine site have been reported to overcome acquired drug resistance, in our study, colchicine-either alone or in combination with miR-22-did not affect TMZ resistance (8). Notably, no prior studies addressing this specific combination were found in the literature.

We determined the IC_{50} value for colchicine to be 5.88 nM in U87 cells on day 3. When comparing this value to those reported in other human cancer cell lines treated with colchicine for the same duration, similar IC_{50} values have been observed; 10 nM in U87 cells and 11 nM in LN-18 cells, 6.3 nM in SKOV3 and 23 nM in A2780 ovarian cancer cell lines, 12.1 nM in MDA-MB-231, 30.3 nM and 10 nM in MCF-7 breast cancer cell lines, 31.4 nM in A549 lung cancer cell line, 9.37 nM in HCT116 colon cancer cell line, and 5 nM in HepG2 hepatocellular carcinoma cell line (37-40). These findings indicate that the IC_{50} value we obtained for colchicine in U87 glioblastoma cells (5.88 nM) falls within the reported range (5-31.4 nM) for other cancer cell lines under similar treatment conditions. This suggests that the GBM cells used in our study display comparable sensitivity to colchicine, supporting its potential as a broadly effective anti-cancer agent across different tumor types. Based on these findings, we used 10 ng/ml colchicine—the dose inducing maximal miR-22-3p expression—for all subsequent analyses, except for the cell viability assay. Notably, there was no significant difference in proliferation between 10 and 100 ng/ml colchicine, suggesting that 10 ng/ml represents the most effective yet least cytotoxic dose. These results are consistent with previous reports showing that low nanomolar concentrations of colchicine exert measurable anti-tumor activity in diverse cancer models, while higher doses do not necessarily confer additional benefits.

This study highlights that miR-22 inhibition increased colony formation capacity, whereas colchicine treatment impaired it in U87 cells. Although there is no available data on colony formation associated with colchicine or colchicine in combination with miR-22 in any cancer type including GBM, a few studies have examined the effects

of colchicine on colony in other cancers. In human head and neck squamous cell carcinoma cell lines, A253 and Detroit562, colony formation capacity decreased by 2- and 5-fold, respectively, following 10 nM colchicine treatment on day 14 (41). In human osteosarcoma cell lines Saos and U2, 30 nM colchicine reduced colony formation by 15- and 6-fold on day 12, respectively (36). In our study, 10 nM colchicine treatment led to 1.5-fold reduction in colony formation in U87 cells on day 14. Compared to other cancer cell lines, colchicine exhibited a milder yet consistent inhibitory effect on colony formation in U87 cells, suggesting potential cell type-specific sensitivity. Regarding the role of miR-22-3p in GBM, previous studies reported 3-fold decrease in colony formation in primary GBM cells, and a 2-fold reduction in U251, LN18 and GSC8-11 GBM cell lines following miR-22-3p transfection, compared to NCs (18, 42, 43). Consistent with these findings, our results showed that colony numbers were 1.5 times higher in miR-22 inhibited U87 cells compared to controls. Moreover, the consistency between viability and colony formation analysis data in this study enhances this study's reliability.

This study highlights that colchicine reduced GBM cell migration independently of miR-22 expression. Although there are no studies in the literature examining the effect of colchicine – or colchicine in combination with miR-22- on GBM migration, a limited number of studies have investigated the impact of colchicine on cell migration in other cancer types. In FaDu cells, a 50% reduction in migration was observed on day 1 following treatment with 10 nM colchicine (35). Similarly, Saos and U2 cells exhibited 40% and 50% reductions in migration, respectively, after 10 nM colchicine treatment on day 2 (36). In gastric cancer, cell migration capacity decreased by 70% and 75% in AGS and NCI-N87 cell lines, respectively, following treatment with 25 nM colchicine on day 1 (44). Compared to these studies reporting 40–75% reductions in migration across cancer cell lines with 10-25 nM colchicine, our finding of a 63% reduction in U87 cell migration with 10 ng/ml (25 nM) colchicine on day 2 demonstrates a comparable anti-migratory effect in GBM.

Our results indicate that colchicine reduces the wound healing capacity of GBM cells independently of miR-22 expression. A previous study reported that miR-22-3p decreased the wound healing capacity of U87 cells by 50% compared to controls on day 3 (16). In our study, following miR-22 inhibitor transfection, the wound healing capacity of U87 cells decreased by 25% compared to control cells on day 2. This discrepancy is likely due to the difference in timing of analysis. Although there is no direct information regarding the wound healing capacity of GBM or other cancer types in association with both colchicine and miR-22, a limited number of studies have investigated the effect of colchicine on wound healing capacity in other cancer types. For instance, treatment with 10 nM colchicine decreased the wound healing capacity of FaDu cells by 25% on day 1 (35). Similarly, our results showed that, 10 nM colchicine reduced the wound healing capacity of U87 cells by 15% on day 2. Although the percentage differences are not striking, they may be attributed to inherent cellular differences the cancer types studied. Furthermore, in this study, it was observed that the migration and wound healing analysis data were parallel, which increases the reliability of the study.

CONCLUSION

In conclusion, the present study investigated the effects of colchicine on GBM cells and its potential interaction with miR-22. Our findings demonstrate that colchicine upregulates miR-22-3p expression in a dose-dependent manner and significantly inhibits cell viability and colony formation in U87 cells. Moreover, while the reduction in cell viability and colony formation appears to be associated with increased miR-22 expression, colchicine impaired migration and wound healing independently of miR-22, suggesting multiple, potentially distinct mechanisms of action. Although the study is limited to a single GBM cell line and an in vitro monolayer culture system, the results provide the first evidence of colchicine-mediated regulation of miR-22 and its functional impact in GBM. These findings contribute to our understanding of colchicine's antitumoral properties and suggest that miR-22 may be a key mediator in certain contexts. Further investigations using multiple GBM cell lines and in vivo models are warranted to validate these findings and explore the therapeutic potential of targeting colchicine–miR-22 pathways in glioblastoma.

Ethics Committee Approval

Commercially available cell lines were used in this study. Therefore, ethical approval is not required.

Informed Consent

All the participants' rights were protected and written informed consents were obtained before the procedures according to the Helsinki Declaration

Author Contributions

Concept – S.K.; Design - S.K.; Supervision - S.K., Öz. Ö., Ö.Ö.; Resources - S.K., Öz. Ö., Ö.Ö.; Materials - S.K., Öz. Ö., Ö.Ö.; Data Collection and/or Processing - S.K., Öz. Ö., Ö.Ö.; Analysis and/ or Interpretation - S.K.; Literature Search - S.K.; Writing Manuscript - S.K.; Critical Review - S.K., Öz. Ö., Ö.Ö.

Conflicts of Interest

The authors have no conflict of interest to declare.

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