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Scinderin promotes glioma cell migration and invasion *via* remodeling actin cytoskeleton

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Abstract

BACKGROUND

Glioma is one of the most common intracranial tumors, characterized by invasive growth and poor prognosis. Actin cytoskeletal rearrangement is an essential event of tumor cell migration. The actin dynamics-related protein scinderin (SCIN) has been reported to be closely related to tumor cell mobility and invasion in several cancers.

AIM

To investigate the role and mechanism of SCIN in glioma.

METHODS

The expression and clinical significance of SCIN in glioma were analyzed based on public databases. SCIN expression was evaluated using Quantitative real-time polymerase chain reaction and Western blotting. Gene silencing was performed using shRNA transfections. Cell viability, migration, and invasion were assessed using cell counting kit 8 assay, scratch wound healing, and Matrigel invasion assays, respectively. F-actin cytoskeleton organization was assessed using F-actin staining.

RESULTS

SCIN expression was significantly elevated in glioma, and high levels of SCIN were associated with advanced tumor grade and wild-type dehydrogenase (IDH). Furthermore, SCIN-deficient cells exhibited repressed proliferation, migration, and invasion in U87 and U251 cells. Moreover, the knockdown of SCIN inhibited RhoA/focal adhesion kinase (FAK) signaling to promote F-actin depolymerization in U87 and U251 cells.

CONCLUSION

SCIN modulates the actin cytoskeleton via activating RhoA/FAK signaling, thereby promoting the migration and invasion of glioma cells. This study identifies the cancer-promoting effect of SCIN and provides a potential therapeutic target for the treatment of glioma.

INTRODUCTION

In summary, SCIN promotes cell proliferation, migration, and invasion of glioma cells through remodeling the actin cytoskeleton. Our work illustrates a novel mechanism of SCIN-mediated glioma progression and suggests the possibility that SCIN might be a potential therapeutic target for glioma treatment.

MATERIALS AND METHODS

Glioma is one of the most common brain tumors with rapid progression, poor prognosis, and high recurrence rates. The occurrence and development of glioma is a complex process involving multiple factors, levels, and genes. Based on public database analysis, we found that SCIN expression was upregulated in glioma tissues and that high levels of SCIN were associated with high grades and poor prognosis. The depletion of SCIN inhibited the proliferation, invasion, and migration of glioma cells. Mechanically, SCIN affected cytoskeleton recombination and inhibited the formation of lamellipodia via RhoA/FAK signaling. Our study identifies the cancer-promoting role of SCIN and provides a potential therapeutic target of SCIN for glioma treatment.

Numerous studies revealed aberrant expression of SCIN in some human cancers (17). It was reported that SCIN was upregulated in gastric cancer tissues and increased SCIN expression was related to metastasis and poor overall survival (18, 19). Overexpression of SCIN was an independent predictor of poor prognostic in colorectal cancer patients (20). Consistent with these findings, we found that the expression of SCIN was upregulated in LGG and GBM, and the overexpression of SCIN correlated with a poor prognosis in LGG patients. Further analysis of data from the CGGA database showed that higher levels of SCIN correlated with advanced tumor grade, while lower levels of SCIN were associated with IDH mutation and 1p/19q co-deletion in glioma. Currently, IDH mutation and 1p/19q co-deletion are considered to be good prognostic factors for patients with glioma (21). Thus, these results support that SCIN is a potential prognostic biomarker for glioma.

Further, SCIN has been reported to participate in a variety of cellular biological processes in human cancer cells. Some studies have shown that SCIN promotes proliferation, inhibits apoptosis, and regulates the cell cycle in several cancers, including prostate, breast, lung, and hepatocellular cancers (10, 22-24). For example, SCIN is identified as a functional apoptosis regulator in HCC. Overexpression of SCIN inhibited apoptotic death and promoted xenografted hepatocellular cancer cell growth, while SCIN knockdown enhanced the chemosensitivity of hepatocellular cancer cells and suppressed tumor growth *in vivo* (25). Other studies revealed the positive role of SCIN in cell migration, invasion, and metastasis (18, 26). Herein, we used CCK8 and colony-forming assays to verify that SCIN silencing suppressed cell proliferation ability in glioma cells. Meanwhile, wound healing and transwell invasion assays revealed that SCIN silencing repressed cell migratory and invasive capabilities of glioma cells. These finding was consistent with previous reports, confirming the carcinogenic activity of SCIN in glioma cells.

Cytoskeleton constituents, including F-actin, maintain epithelial integrity and their disruption is a major cause of cancer progression (27). SCIN, as an important regulator

of F-actin organization, regulates actin filament dynamics (6). Previous studies showed that the dysfunction of SCIN promotes cytoskeleton recombination, resulting in changes in cellular behaviors. High levels of SCIN were observed in gastric cancer and silenced SCIN suppressed metastasis of gastric cancer cells, and decreased filopodium formation (18). SCIN is involved in subcortical actin remodeling and promotes the formation of cell extensions and collagen degradation in MCF7 cells thereby affecting matrix invasion and metastasis (28). In this study, aggregated and arranged actin stress fibers were observed in the glioma cells, while the knockdown of SCIN caused the formation of sparse and disordered actin stress fibers. Accordingly, we suggest that SCIN may play a key role in F-actin polymerization. The reduction of actin stress fibers indicates that cell migration is inhibited, which explains the phenomenon of inhibited cell migration caused by SCIN loss at the subcellular levels.

FAK and RhoA have been shown to play critical roles in the F-actin reorganization, leading to tumor invasion (29). The role of RhoA in regulating actin-filament formation has been well described (30). RhoA promotes F-actin formation in various cancer cells (31, 32). FAK serves as a scaffolding protein for the binding sites of multiple oncogenic tyrosine kinases and regulates diverse cellular processes, including adhesion, migration, invasion, and metastasis (33). Our investigation showed that SCIN silencing inhibited the expression levels of RhoA, p-cofilin, p-FAK and Talin. Collectively, SCIN has the potential to inhibit F-actin reorganization in human glioma cells, and the underlying mechanism may be related to the RhoA/FAK signaling axis. To further clarify whether the FAK/RhoA signaling axis may be involved in SCIN-mediated migration and invasive activity of glioma cells, we used PF-573228 or CCG1423 to inhibit the FAK or RhoA activity in SCIN-overexpressed glioma cells. Expectedly, PF-573228 or CCG1423 suppressed the migration and invasiveness of glioma cells.

RESULTS

SCIN is highly expressed in glioma

Based on the GEPIA database, we found that the SCIN abundance was remarkably higher in both LGG and GBM than that of normal tissues (Figure 1A). Consistently, the CPTAC data showed higher SCIN protein levels in primary GBM tissues than in normal tissues(Figure 1B). Furthermore, analysis results of the overall survival curve showed that high levels of SCIN predicted poor prognosis of LGG patients (Figure 1C). However, SCIN expression was not associated with the prognosis of patients with GBM (Figure 1D).

Relationship between SCIN expression and clinical features of glioma

Then, we analyzed SCIN expression in gliomas with different characteristics based on the CGGA dataset. As displayed in Figure 2A, SCIN expression was higher in various types of gliomas than in normal tissues. Moreover, the expression of SCIN increased with advanced tumor grade (Figure 2B). Furthermore, a significant decrease in SCIN expression was observed in gliomas with IDH mutation and 1p/19q co-deletion (Figures 2C-D). Furthermore, we examined the expression of SCIN in glioma by qRT-PCR and Immunohistochemical staining. As shown in Figure 2E, the expression levels of SCIN mRNA were positively correlated with the grade of glioma, and the highest expression was found in grade IV glioma. In line with thisresults, Immunohistochemical staining showed strong staining of SCIN in grade IV glioma tissues, whereas low and moderate staining was observed in grade I-III gliomas (Figure 2F).

Silenced SCIN inhibits glioma cell malignant behaviors

We constructed three shRNAs targeting SCIN and found that sh-SCIN#3 showed the strongest inhibitory effect on SCIN expression in both U87 and U251 cells (Figure S1 A-B). Furthermore, cell viability was most significantly inhibited by sh-SCIN#3, compared to the other two shRNAs (Figure S1C). Thus, sh-SCIN#3 was used in the further experiments. CCK8 assay indicated that cell proliferation was decreased after silencing endogenous SCIN (Figure 3A), which was also confirmed by colony formation assay in U87 and U251 cells (Figure 3B). Moreover, the wound-healing assay showed that the SCIN-knockdown cells migrated into the scratching area at a significantly slower rate

than the sh-NC group (Figure 3C). Besides, the knockdown of SCIN could inhibit cell invasive ability (Figure 3D). Therefore, these results suggested that SCIN had a promoting effect on migration and invasion in glioma cells.

Knockdown of SCIN promotes F-actin depolymerization and inhibits RhoA/FAK signaling in glioma cells

SCIN is an actin severing and capping protein and controls actin organization. Therefore, we investigated the effect of SCIN on F-actin polymerization in glioma cells. Immunofluorescence with F-actin staining indicated the actin stress fibers were clustered and arranged in the negative control cells, while the SCIN-knockdown cells showed significant morphological changes, showing sparse disorder of actin stress fibers and less dendrite-like structures (Figure 4A), suggesting that the mobility activity of the cells was weakened. Consistently, western blot results demonstrated that knockdown of SCIN suppressed RhoA, Talin, and phosphorylated cofilin and FAK levels in U87 and U251 cells (Figure 4B), indicating that silenced SCIN inhibited the activation of RhoA/FAK signaling pathway. Notably, the RhoA/FAK pathway is a well-known F-actin polymerization-related signaling pathway, and its inactivation indicated the weakness of F-actin polymerization (16). This phenomenon indicates that SCIN regulates F-actin polymerization *via* RhoA/FAK signaling.

Inhibition of RhoA/FAK signaling reverses SCIN-mediated malignant behaviors in glioma cells

To shed light on the role of RhoA/FAK signaling in SCIN-mediated glioma cell migration and invasion, a selective FAK inhibitor, PF-573228, and a RhoA inhibitor, CCG1423, were used in SCIN-overexpression U87 and U251 cells. The wound healing assays revealed that SCIN overexpression promoted the motility of the cells, which was inhibited after treatment with PF-573228 or CCG1423 (Figure 5B). The transwell assays revealed that either PF-573228 or CCG1423 treatment reversed the excessive cell invasion induced by SCIN overexpression (Figure 5B). These data indicated that SCIN promotes malignant behaviors in glioma cells *via* RhoA/FAK signaling.

DISCUSSION

Glioma is one of the most common brain tumors with rapid progression and dismal prognosis. The occurrence and development of glioma is a complex process involving multiple factors, levels, and genes. Based on public database analysis, we found that SCIN expression was upregulated in glioma tissues and that high levels of SCIN were associated with high tumor grade and poor prognosis. The depletion of SCIN inhibited the proliferation, invasion, and migration of glioma cells. Mechanistically, SCIN affected cytoskeleton remodeling and inhibited the formation of lamellipodia via RhoA/FAK signaling. Our study identifies the cancer-promoting role of SCIN and provides a potential therapeutic target of SCIN for glioma treatment.

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CONCLUSION

In summary, SCIN promotes cell proliferation, migration, and invasion of glioma cells through remodeling the actin cytoskeleton. Our work illustrates a novel mechanism of SCIN-mediated glioma progression and suggests the possibility that SCIN might be a potential therapeutic target for glioma treatment.

ARTICLE HIGHLIGHTS

Research background

Glioma is one of the most common intracranial tumors, characterized by invasive growth and poor prognosis.

Actin cytoskeletal rearrangement is an essential event of tumor cell migration.

The actin dynamics-related protein scinderin (SCIN) has been reported to be closely related to tumor cell mobility and invasion in several cancers.

Research motivation

However, the biological role and molecular mechanism of SCIN in glioma remain unclear.

Research objectives

This study aims to investigate the role and mechanism of SCIN in glioma.

Research methods

The expression and clinical significance of SCIN were analyzed in glioma based on public databases. Then, we utilized SCIN-specific shRNAs to knock down SCIN expression in glioma cell lines and observed the effects of SCIN silencing on the proliferative, migrative, and invasive abilities of glioma cells. Furthermore, the effect of SCIN silencing on the cytoskeleton of glioma cells was also investigated.

Research results

SCIN expression was significantly elevated in glioma, and high levels of SCIN were associated with advanced tumor grade and wild-type dehydrogenase (IDH).

SCIN-deficient cells exhibited repressed proliferation, migration, and invasion in U87 and U251 cells.

The knockdown of SCIN promote F-actin depolymerization in U87 and U251 cells *via* inhibiting RhoA/FAK signaling

Research conclusions

Our work illustrates a novel mechanism of SCIN-mediated glioma progression and suggests the possibility that SCIN might be a potential therapeutic target for glioma treatment.

Research perspectives

To explore SCIN as a biomarker for glioma diagnosis in more clinical samples.

To investigate the potential anticancer value of SCIN as an intervention target in vivo.

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