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Downregulation of SCAI enhances glioma cell invasion and stem cell like phenotype by activating wnt/β-catenin signaling

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ABSTRACT

SCAI (suppressor of cancer cell invasion), has been recently characterized as a novel tumor suppressor that inhibits the invasive migration of several human tumor cells. However, the expression pattern, biological role and molecular mechanism of SCAI in human glioma remain unknown. In this study, we found that levels of SCAI protein and mRNA expression were significantly downregulated in glioma tissues and cell lines. Overexpression of SCAI inhibited, but silencing of SCAI robustly promoted the invasive and cancer stem cell-like phenotypes of glioma cells. Furthermore, we demonstrated that SCAI downregualtion activated the wnt/β-catenin signaling, and blockade of the wnt/β-catenin pathway abrogated the effects of SCAI downregulation on glioma cell aggressiveness. Taken together, our results provide the first demonstration of SCAI downregulation in glioma, and its downregulation contributes to increased glioma cell invasion and self-renewal by activating the wnt/β-catenin pathway.

1. Introduction

Glioma is one of the most malignant and lethal human cancers with a median survival being only approximately 12–15 months [1]. Recent studies revealed that the robust ability of migration through cerebrum and self renewal are the two most significant glioma phenotypic features, which might contribute to destruction of functional brain tissue, incomplete surgical resection of the tumor mass and extremely high frequency of relapse [2–4]. However, the molecular mechanisms of glioma aggressiveness remains poorly understood, and thus far, no effective prognostic or therapeutic targets have been developed. Thus, delineation of the mechanisms that regulate cell invasion and self-renewal in glioma may allow the identification of novel targets that could serve as possible targets for therapeutic intervention.

The wnt/β-catenin signaling pathway is constitutive activated in a variety of human cancers, including glioma [5]. The wnt/β-catenin signaling plays a critical role in tumorigenesis by transcriptional regulation of its downstream genes, such as MYC [6], MMP7 [7], Snail [8] and Sox9 [9], thus leading to cell proliferation, invasion and cancer stem cell property maintenance [10,11]. At this point, targeting the wnt/β-catenin signaling might be a promising strategy for glioma therapy. Unlike colorectal cancer, in which hyperactivation of β-catenin are frequently found as a result of mutational loss of the adenomatous polyposis coli (APC) gene, or stabilizing mutations in the β-catenin gene itself [12], APC loss or β-catenin gene mutation was rarely found in glioma [13]. Therefore, delineation of the mechanisms that regulate wnt/β-catenin signaling may provide new clues for the development of targeted cancer therapies for glioma.

SCAI is recently characterized as a putative tumor suppressor that inhibits the invasive migration of human tumor cells through the transcriptional co-repression of MAL/SRF signaling [14,15]. Consistently, reduced SCAI expression was found in several tumors [15]. Notably, SCAI RNA levels are abundant in the brain [14]. However, its expression and role in glioma remains unknown.

In the current study, we found that SCAI was robustly reduced in gliomas tissues and cell lines. Knockdown of SCAI promotes, and ectopic expression of SCAI inhibits the invasive and cancer stem cell-like phenotype of glioma. Furthermore, we demonstrated that SCAI downregulation led to transcriptional activation of the wnt/β-catenin pathway. Taken together, our results suggest that SCAI act as a tumor suppressor and its downregulation may play an important role in the development and progression of glioma.

2. Materials and methods

2.1. Tissues and cells

Glioma tissues were obtained from the SunYat-Sen Memorial Hospital of Sun Yat-sen University. Normal brain tissues were

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obtained from individuals who died in traffic accidents and confirmed to be free of any pre-existing pathologically detectable conditions. The approval from the Institutional Research Ethics Committee and donators' consents were obtained.

NHA (Normal human astrocyte, Sciencell) were cultured under the condition as the manufacture instructed. Glioma cell lines LN444, SNB19, U251MG, LN18, U118MG, A172 and U87MG, obtained from ATCC, were routinely maintained in DMEM medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (HyClone, Logan, UT).

2.2. Plasmids and oligonucleotides

pMSCV/SCAI overexpressing human SCAI was generated by subcloning the PCR-amplified human SCAI coding sequence into pMSCV-puro-retro vector. To silence endogenous SCAI, two RNAi oligonucleotides were cloned into the pSuper-retro-puro vector to generate pSuper-retro-SCAI-RNAi(s), respectively. The shRNA sequences were as following: shRNAi#1: CGGAUGUUACAAGCUCUGGAA; shRNAi#2: CCCAGAUGAAUAAACCAGGAA. Retroviral production was performed in 293T cells. Stable cell lines expressing SCAI or SCAI shRNA(s) were selected for 10 days with 0.5 μg/ml puromycin 48 h after infection. After 10-day selections, the cell lysates prepared from the pooled population of cells in sample buffer were fractionated on SDS–PAGE for the detection of SCAI protein level.

2.3. Western blotting

Cells were harvested in cell lysis buffer (Cell Signaling Technology; Cat#: 9803) and heated for 5 min at 100 °C. Equal quantities of denatured protein samples were resolved on 10% SDS–polyacrylamide gels, and then transferred onto polyvinylidene difluoride membranes (Roche). After blocking with 5% non-fat dry milk in Tris-buffered saline/0.05% Tween 20 (TBST), the membrane was incubated with a specific primary antibody, followed by the horseradixh peroxidase-conjugated secondary antibody. Proteins were visualized using ECL reagents (Pierce). The anti-SCAI and anti-α-Tubulin antibodies were purchased from abcam (Cambridge, MA).

2.4. Real-time PCR

Total mRNA from cultured cells and fresh surgical glioma tissues was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's manual. Real-time PCR was performed using the Applied Biosystems 7900 Sequence Detection system. Expression data were normalized to the mean of housekeeping gene GAPDH to control the variability in expression levels. The primers used are as following: SCAI-up, CTGACTGGCACAGTGGAGAA; SCAI-dn, TCTTTCCCCTTGAGATTGTGA; Oct4-up, GTGGAGGAAGCTGACAACAA; Oct4-dn, GGTTCTCGATACTGGTTCGC; Nanog-up, GATTTGTGGGCCTGAAGAAA; Nanog-dn, ATGGAGGAGGGAAGAGGAGA; Sox2-up, AACCCCAAGATGCACAACT; Sox2-dn, GCTTAGCCTCGTCGATGAAC; CD133-up, CCATTGGCATTCTCTTTGAA; CD133-dn, TTTGGATTCATATGCCTTCTGT; Ascl2-up, GGCACCAACACTTGGAGATT; Ascl2-dn, CCCTCCAGCAGCTCAAGTTA; CCND1-up, TCCTCTCCAAAATGCCAGAG; CCND1-dn, GGCGGATTGGAAATGAACTT; Frizzled 7-up, CGCCTCTGTTCGTCTACCTC; Frizzled 7-dn, GTCGTGTTTCATGATGGTGC; LGR5-up, CAGCGTCTTCACCTCCTACC; LGR5-dn, GTTTCCCGCAAGACGTAACT; MMP7-up, GAGCTACAGTGGGAACAGGC; MMP7-dn, GCATCTCCTTGAGTTTGGCT; Snail-up, CCTTCTCTAGGCCCTGGCT; Snail-dn, AGGTTGGAGCGGTCAGC; Sox9-up, GACGCTGGGCAAGCTCT; Sox9-dn, GTAATCCGGGTGGTCCTTCT; Twist-up, GTCCGCGTCCCACTAGC; Twist-dn, TCCATTTTCTCCTTCTCTGGAA; GAPDH-up, AAGGTGAAGGTCGGAGTCAA; GAPDH-dn, AATGAAGGGGTCATTGATGG.

2.5. Wound healing assay

Cells were seeded on six-well plates with DMEM containing 10% fetal bovine serum (FBS) and grown to confluence. The cells were scratched with a sterile 200-AL pipette tip to create artificial wounds. At 0 and 24 h after wounding, phase-contrast images of the wound healing process were photographed with a 10× objective lens. Eight images per treatment were analyzed to determine averaging position of the migrating cells at the wound edges.

2.6. Transwell matrix penetration assay

Cells (1×10^4) to be tested were plated on the top side of polycarbonate transwell filter (with Matrigel) in the upper chamber of the BioCoat™ Invasion Chambers (BD, Bedford, MA) and incubated at 37 °C for 24 h. Invaded cells on the lower membrane surface were fixed in 1% paraformaldehyde, stained with crystal violet, and counted (Ten random 100×fields per well). Three independent experiments were performed and the data are presented as mean ± standard deviation (SD).

2.7. Luciferase assays

Cells (2×10^4) were seeded in triplicates in 48-well plates and cultured for 24 h. One hundred nanogram of TOP flash or FOP flash luciferase plasmid were transfected into indicated cells using the Lipofectamine 2000 reagent (Invitrogen Co., Carlsbad, CA) according to the manufacturer's recommendation. Luciferase signals were measured 36 h after transfection using the Dual Luciferase Reporter Assay Kit (Promega, Madison, WI) according to the manufacturer's protocol. The results were presented as the relative TOP/FOP ratio.

2.8. Chromatin immunoprecipitation (ChIP) assay

Cells (2×10^6) in a 100-mm culture dish were treated with 1% formaldehyde to cross-link proteins to DNA. The cell lysates were sonicated to shear DNA to sizes of 300–1000 bp. Equal aliquots of chromatin supernatants were incubated with 1 μg of an anti-β-catenin, or an anti-IgG antibodies (Millipore, Billerica, MA) overnight at 4 °C with rotation. After reverse cross-link of protein/DNA complexes to free DNA, PCR was performed.

2.9. Statistical analysis

The two-tailed Student's *t*-test was used to evaluate the significance of the differences between two groups of data in all pertinent experiments. A P values <0.05 was considered significant.

3. Results

3.1. SCAI is robustly reduced in gliomas

To investigate the biological role of SCAI in glioma progression, we first examined the expression level of SCAI in gliomas. As shown in Fig. 1A and B, Western blotting analysis revealed that SCAI was differentially downregulated in glioma patient tissues and cell lines, compared with those in normal brain tissues and NHA cells respectively. Meanwhile, using the real-time PCR analysis, we found that the transcript levels of SCAI were also reduced in glioma tissues and cell lines (Fig. 1C and D). Consistently, publicly

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