Molecular Therapy
Original Article

Intra-articular Delivery of Antago-miR-483-5p Inhibits Osteoarthritis by Modulating Matrilin 3 and Tissue Inhibitor of Metalloproteinase 2

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MicroRNAs (miRNAs) are emerging as important regulators in osteoarthritis (OA) pathogenesis. In our study, a real-time PCR assay revealed that miR-483-5p was upregulated in articular cartilage from OA patients and experimental OA mice induced by destabilization of the medial meniscus compared to their controls. Overexpression of miR-483-5p by intra-articular injection of lentivirus LV3-miR-483-5p significantly enhanced the severity of experimental OA. Consequently, we synthesized antago-miR-483-5p to silence the endogenous miR-483-5p and delivered it intra-articularly, which revealed that antago-miR-483-5p delayed the progression of experimental OA. To investigate the functional mechanism of miR-483-5p in OA development, we generated doxycycline-inducible miR-483 transgenic (TG483) mice. TG483 mice exhibited significant acceleration and increased severity of OA, and age-related OA occurred with higher incidence and greater severity in TG483 mice compared with their controls. Furthermore, our results revealed miR-483-5p directly targeted to the cartilage matrix protein matrilin 3 (Matn3) and tissue inhibitor of metalloproteinase 2 (Timp2) to stimulate chondrocyte hypertrophy, extracellular matrix degradation, and cartilage angiogenesis, and it consequently initiated and accelerated the development of OA. In conclusion, our findings reveal an miRNA functional pathway important for OA development. Targeting of miR-483-5p by intra-articular injection of antago-miR-483-5p represents an approach that could prevent the onset of OA and delay its progression.

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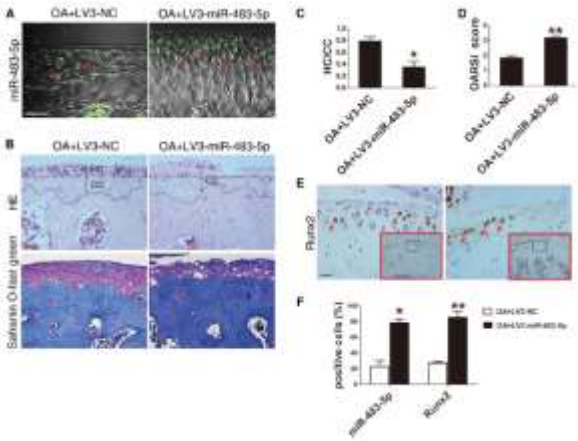

Methods

With regard to lentivirus (GenePharma) injection, 10 mL lentivirus- mediated miR-483-5p, siTimp2, or NC, Matn3, TIMP2, and corresponding negative controls were injected into the knee joint34 of male mice using a 33G needle and a micro-syringe. For the antago-miR-483-5p injection, 250 mM antago-miR-483-5p or antago-mir NC (GenePharma) were injected. All experimental mice were injected on day 7 and day 14 after surgery in the OA model. Knee joints were harvested 5 weeks later.

The miRNA mimics and miRNA inhibitors were synthesized by GenePharma.

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Cell Death & Disease

ARTICLE Open Access

Circular RNA circSLC25A16 contributes to the glycolysis of non-small-cell lung cancer through epigenetic modification

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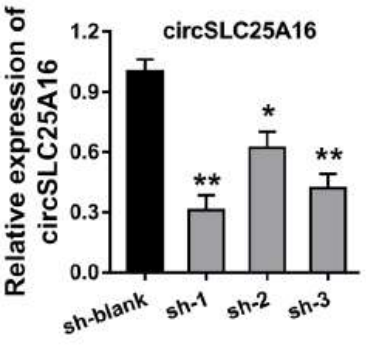
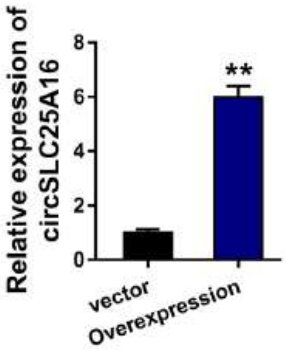
Abstract

Growing evidence has highlighted the roles of circular RNAs (circRNAs) in non-small-cell lung cancer (NSCLC), however, their roles in NSCLC glycolysis remains poorly understood. CircRNAs microarray profiles discovered a novel exon-derived circRNA, circSLC25A16 (hsa_circ_0018534), in NSCLC tissue samples. In NSCLC samples, high-expression of circSLC25A16 was associated with unfavorable prognosis. Cellular experiments revealed that circSLC25A16 accelerated the glycolysis and proliferation of NSCLC cells. Besides, circSLC25A16 knockdown repressed the in vivo growth by xenograft assays. RNA-fluorescence in situ hybridization (RNA-FISH) illustrated that circSLC25A16 and miR-488-3p were both located in cytoplasm. Mechanistic experiments demonstrated that circSLC25A16 interacts with miR-488-3p/HIF-1α, which activates lactate dehydrogenase A (LDHA) by facilitating its transcription. Collectively, present research reveals the crucial function of circSLC25A16 on NSCLC glycolysis through miR-488-3p/HIF-1α/LDHA, suggesting the underlying pathogenesis for NSCLC and providing a therapeutic strategy for precise treatment.





Cell Death & Disease

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Methods

For circRNA silencing, the sh-circSLC25A16 and sh-NC were constructed by GenePharma Biotech (Shanghai, China). Cells were transfected with the recombinant lentiviral transduction particles (GenePharma).

Cy3-labeled probe sequences for circSLC25A16 and FAM-labeled probe for miR-488-3p were constructed by Genepharma (Shanghai, China). RNA FISH were performed for analysis of the co-localization of circSLC25A16 and miR-488-3p in NSCLC cells using fluorescent in situ hybridization kit (Genepharma) according to the manufacturer's protocol.

Mutants were also constructed. 293T cells were co-transfected with the psicheck2-based plasmids with miR-488-3p mimics or inhibitors (GenePharma, Shanghai, China).

Plasmids

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Integrin alpha 7 correlates with poor clinical outcomes, and it regulates cell proliferation, apoptosis and stemness via PTK2-PI3K-Akt signaling pathway in hepatocellular carcinoma

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ABSTRACT

This study aimed to evaluate the correlation of integrin alpha 7 (ITGA7) with clinical outcomes and its effect on cell activities as well as stemness in hepatocellular carcinoma (HCC). HCC tumor tissues and paired adjacent tissues from 90 HCC patients were obtained and ITGA7 expression was detected using immunohistochemistry assay. Cellular experiments were conducted to examine the effect of ITGA7 on cell activities, astemness via ITGA7 ShRNA transfection, and compensation experiments were further performed to test whether ITGA7 functioned via regulating PTK2-PI3K-AKT signaling pathway. ITGA7 was overexpressed in tumor tissues compared with paired adjacent tissues and its high expression was correlated with larger tumor size, vein invasion and advanced Barcelona Clinic Liver Cancer stage, and it also independently predicted worse overall survival in HCC patients. In cellular experiments, ITGA7 was upregulated in SMMC-7721, Hep G2, HuH-7 and BEL-7404 cell lines compared with normal human liver cells HL-7702. ITGA7 knockdown suppressed cell proliferation but promoted apoptosis, and it also downregulated CSCs markers (CD44, CD133 and OCT-4) as well as PTK2, PI3K and AKT expressions in SMMC-7721 and Hep G2 cell lines. ITGA7 overexpression promoted cell proliferation but inhibited apoptosis, and it also upregulated CSCs markers in HL-7702 cells. Further compensation experiments verified that ITGA7 regulated cell proliferation, apoptosis and CSCs markers via PTK2-PI3K-Akt signaling pathway. ITGA7 negatively associates with clinical outcomes in HCC patients, and it regulates cell proliferation, apoptosis and CSCs markers via PTK2-PI3K-Akt signaling pathway.

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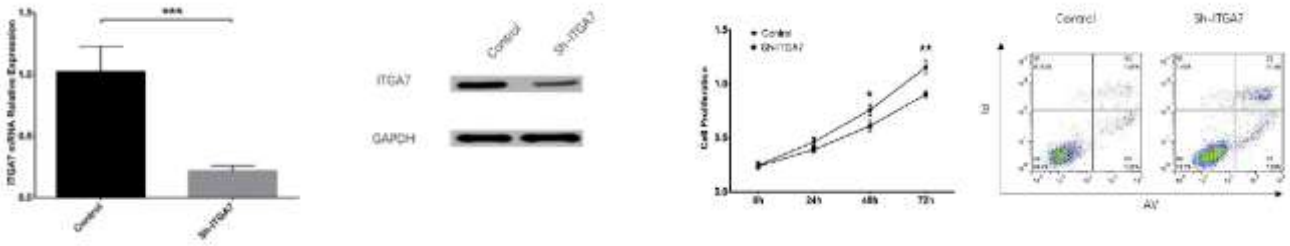

Methods

ITGA7 ShRNA and scrambled control ShRNA were constructed using pGPU6 plasmids (GenePharma, China) by Shanghai GenePharma Bio-Tech Company (Shanghai, China).
ITGA7 overexpression and control overexpression plasmids were constructed using pEX-2 plasmids (GenePharma, China) by Shanghai GenePharma Bio-Tech Company (Shanghai, China),.

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PABPC1-induced stabilization of BDNF-AS inhibits malignant progression of glioblastoma cells through STAU1-mediated decay

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Abstract

Glioblastoma is the most common and malignant form of primary central nervous tumor in adults. Long noncoding RNAs (lncRNAs) have been reported to play a pivotal role in modulating gene expression and regulating human tumor's malignant behaviors. In this study, we confirmed that lncRNA brain-derived neurotrophic factor antisense (BDNF-AS) was downregulated in glioblastoma tissues and cells, interacted and stabilized by polyadenylate-binding protein cytoplasmic 1 (PABPC1). Overexpression of BDNF-AS inhibited the proliferation, migration, and invasion, as well as induced the apoptosis of glioblastoma cells. In the in vivo study, PABPC1 overexpression combined with BDNF-AS overexpression produced the smallest tumor and the longest survival. Moreover, BDNF-AS could elicit retina and anterior neural fold homeobox 2 (RAX2) mRNA decay through STAU1-mediated decay (SMD), and thereby regulated the malignant behaviors glioblastoma cells. Knockdown of RAX2 produced tumor-suppressive function in glioblastoma cells and increased the expression of discs large homolog 5 (DLG5), leading to the activation of the Hippo pathway. In general, this study elucidated that the PABPC1-BDNF-AS-RAX2-DLG5 mechanism may contribute to the anticancer potential of glioma cells and may provide potential therapeutic targets for human glioma.

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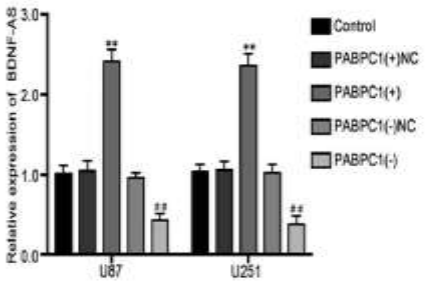
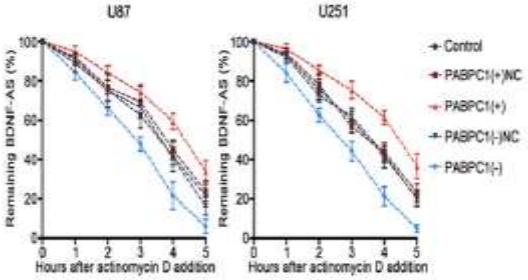
Methods





The short-hairpin RNA directly against human BDNFAS, PABPC1, RAX2, or DLG5 gene and their nontargeting sequences were ligated into pGPU6/ GFP/Neo vectors (GenePharma, Shanghai, China), respectively.

Human BDNF-AS gene and the negative control were ligated into pGCMV/MCS/IRES/EGFP/Neo vector (GenePharma, Shanghai, China).

BDNF-AS full-length and RAX2 3'-UTR sequences were amplified by PCR and cloned in the pmirGlo Dualluciferase Vector (Promega, Madison, USA) to construct luciferase reporter vector (BDNF-AS-Wt and RAX2- Wt) (GenePharma, Shanghai, China).

Human full-length RAX2 was constructed in pEX3 vector (GenePharma, Shanghai, China).