# siRNA synthesis

Biology of Human Tumors

Clinical Cancer Research

## Long Noncoding RNA *NEAT1*, Regulated by the EGFR Pathway, Contributes to Glioblastoma Progression Through the WNT/β-Catenin Pathway by Scaffolding EZH2

**Abstract**

**Purpose:** Long noncoding RNAs have been implicated in gliomagenesis, but their mechanisms of action are mainly undocumented. Through public glioma mRNA expression data sets, we found that *NEAT1* was a potential oncogene. We systematically analyzed the clinical significance and mechanism of *NEAT1* in glioblastoma.

**Experimental Design:** Initially, we evaluated whether *NEAT1* expression levels could be regulated by EGFR pathway activity. We subsequently evaluated the effect of *NEAT1* on the WNT/β-catenin pathway and its target binding gene. The animal model supported the experimental findings.

**Results:** We found that *NEAT1* levels were regulated by EGFR pathway activity, which was mediated by STAT3 and NFκB (p65) downstream of the EGFR pathway. Moreover, we found that *NEAT1* was critical for glioma cell growth and invasion by increasing β-catenin nuclear transport and downregulating ICAT, GSK3B, and Axin2. Taken together, we found that *NEAT1* could bind to EZH2 and mediate the trimethylation of H3K27 in their promoters. *NEAT1* depletion also inhibited GBM cell growth and invasion in the intracranial animal model.

**Conclusions:** The EGFR/*NEAT1*/EZH2/β-catenin axis serves as a critical effector of tumorigenesis and progression, suggesting new therapeutic directions in glioblastoma. *Clin Cancer Res; 24(3); 684–95.*


**Clinical Cancer Research**
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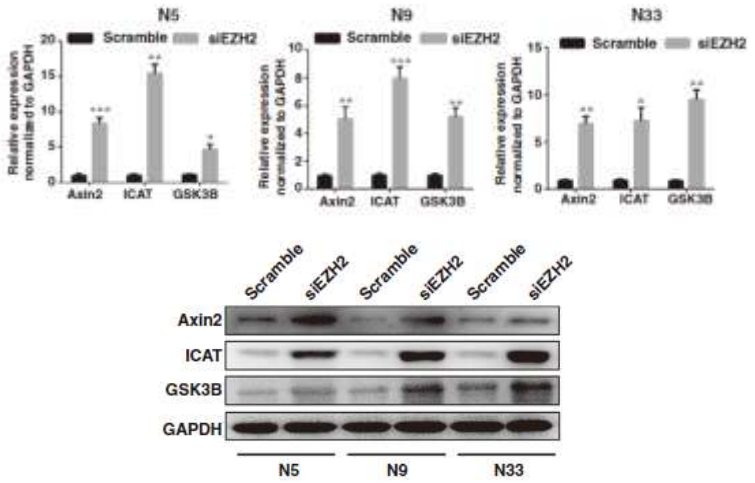


## Methods

Lentivirus such as EGFRvIII, HOTAIR, HOTAIR 50 domain, and the HOTAIR 50 domain mutant were purchased from Shanghai Genepharma.
SiRNAs for EZH2 were purchased from Shanghai Genepharma.
SgRNAs for NEAT1 were purchased from Shanghai Genepharma.

nature structural & molecular biology

ARTICLES

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# APOBEC3 induces mutations during repair of CRISPR-Cas9-generated DNA breaks

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**The APOBEC-AID family of cytidine deaminase prefers single-stranded nucleic acids for cytidine-to-uridine deamination. Single-stranded nucleic acids are commonly involved in the DNA repair system for breaks generated by CRISPR-Cas9. Here, we show in human cells that APOBEC3 can trigger cytidine deamination of single-stranded oligodeoxynucleotides, which ultimately results in base substitution mutations in genomic DNA through homology-directed repair (HDR) of Cas9-generated double-strand breaks. In addition, the APOBEC3-catalyzed deamination in genomic single-stranded DNA formed during the repair of Cas9 nickase-generated single-strand breaks in human cells can be further processed to yield mutations mainly involving insertions or deletions (indels). Both APOBEC3-mediated deamination and DNA-repair proteins play important roles in the generation of these indels. Therefore, optimizing conditions for the repair of CRISPR-Cas9-generated DNA breaks, such as using double-stranded donors in HDR or temporarily suppressing endogenous APOBEC3s, can repress these unwanted mutations in genomic DNA.**

## Nature

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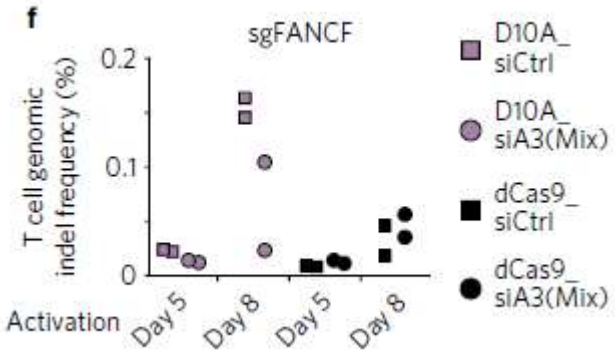


## Methods

Control siRNA (no target in mammalian cells, A06001),
siRNAs against APOBEC3B (5′-GUGAUUAAUUGGCUCCAUA-3′),
APOBEC3C (5′-CAAUGUAUCCAGGCACAUU-3′),
APOBEC3F (5′-GACGAUGAAGAAUUUGCAU-3′),
APOBEC3H(5′-CUGUAAAUCCAGCCGGGUA-3′) and
MRE11 (5′-CACUUCAAAGACAGAUCAA-3′) were synthesized by GenePharma (stable siRNA).

Gan et al. Molecular Cancer (2020) 19:45
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Molecular Cancer

RESEARCH Open Access

# CircMUC16 promotes autophagy of epithelial ovarian cancer via interaction with ATG13 and miR-199a

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**Abstract**

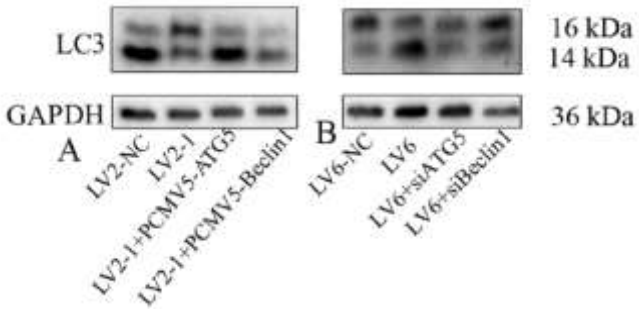
**Background:** Circular RNA (circRNA) has been proven to play a significant role in multiple types of cancer. However, the expression and role of circRNAs in epithelial ovarian cancer (EOC) remains elusive.

**Methods:** CircRNA and mRNA expression profiles of EOC were screened with sequencing analysis. Gene silencing and over-expression were used to study circRNA function. Cell proliferation and Matrigel invasion assays were used to detect cell proliferation and invasion, respectively. The expression of circRNAs, mRNAs and miRNAs was detected using qPCR. The location of circRNAs was detected using FISH. The expression of proteins was detected using western blot and immunohistochemistry.

**Results:** CircMUC16 had increased expression in EOC tissues as compared to healthy ovarian tissues. The expression of circMUC16 was linked to the progression in stage and grade of EOC. Hence, silencing circMUC16 suppressed autophagy flux of SKOV3 cells. In contrast, ectopic expression of circMUC16 promoted autophagy flux of A2780 cells. CircMUC16-mediated autophagy exacerbated EOC invasion and metastasis. Mechanistically, circMUC16 could directly bind to miR-199a-5p and relieve suppression of target Beclin1 and RUNX1. In turn, RUNX1 elevated the expression of circMUC16 via promotion of its transcription. CircMUC16 could directly bind to ATG13 and promote its expression.

**Conclusion:** This study demonstrated that circMUC16 regulated Beclin1 and RUNX1 by sponging miR-199a-5p. The data suggested that circMUC16 could be a potential target for EOC diagnosis and therapy.

**Keywords:** CircMUC16, Autophagy, Beclin1, RUNX1, miR-199a-5p




## Molecular Cancer

(2020) 19:45

## Methods

The circMUC16-lentiviral expression vector (named LV6) were purchased from Genepharma. MIR199A mimics and inhibitors were synthesized by Genepharma. We constructed pCMV5-Beclin1 for overexpression of Beclin1. The siRNA was synthesized by Genepharma (Shanghai, China). Biotin-labelled circMUC16 probe was purchased from Genepharma.

The primers were purchased from Genepharma.

FITClabeled probe against miR-199a were obtained from Genepharma.

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ORIGINAL ARTICLE

# DLX6-AS1/miR-204-5p/OCT1 positive feedback loop promotes tumor progression and epithelial–mesenchymal transition in gastric cancer

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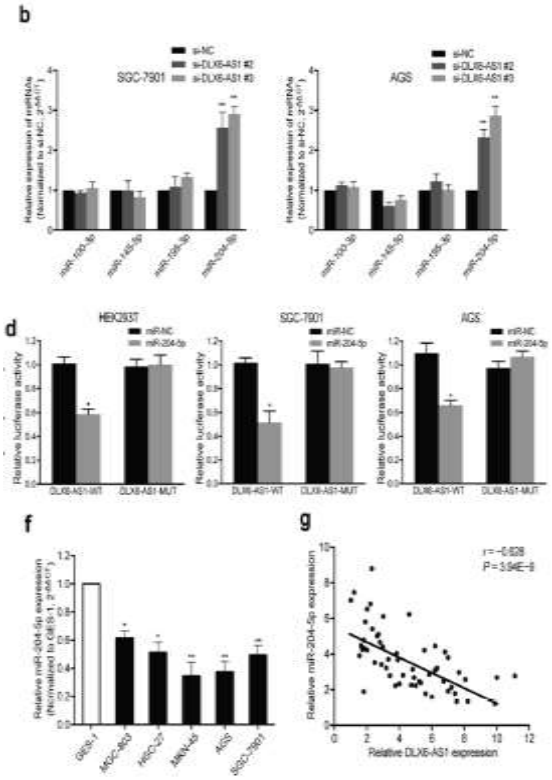
## Abstract

**Background** Accumulating evidence indicates that long non-coding RNAs (lncRNAs) participate in progression of gastric cancer (GC). Nevertheless, the function and expression level of DLX6-AS1 in GC remain unknown.

**Methods** We explored the sequencing data of DLX6-AS1 downloaded from The Cancer Genome Atlas. The expression of DLX6-AS1, miR-204-5p and OCT1 in 56 GC patients and GC cell lines was quantified by qRT-PCR and western blotting. Furthermore, we performed in vitro functional assays to assess proliferation, invasion and migration of GC cells by knock-down of DLX6-AS1. The expression level of epithelial–mesenchymal transition (EMT)-related genes was also determined by qRT-PCR and western blotting. Actin remodeling was detected by F-actin phalloidin staining. The luciferase reporter assay and chromatin immunoprecipitation assay was utilized to confirm the bioinformatic prediction. The function of the DLX6-AS1/miR-204-5p/OCT1 axis in GC proliferation was clarified by rescue assays.

**Results** We first demonstrated that DLX6-AS1 was upregulated in GC tissues and cell lines and was associated with T3/T4 invasion, distant metastasis and poor clinical prognosis. Further functional analysis showed that downregulation of DLX6-AS1 inhibited GC cell proliferation, migration, invasion and EMT in vitro. Mechanistic investigation indicated that DLX6-AS1 acted as a cancer-promoting competing endogenous RNA (ceRNA) by binding miR-204-5p and upregulating OCT1. Moreover, the transcription factor OCT1 was confirmed to enhance DLX6-AS1 expression by targeting the promoter region.

**Conclusions** This study revealed that OCT1-induced DLX6-AS1 promoted GC progression and the EMT via the miR-204-5p/OCT1 axis, suggesting that this lncRNA might be a promising prognostic biomarker and therapeutic target for GC.




## Gastric Cancer

(2020) 23:212–227

## Methods

MiRNA Quantitation Kit were purchased from GenePharma.

According to the manufacturers' protocol, all the small interfering RNAs (siRNAs) (GenePharma) including siRNADLX6-AS1 (#1, #2, #3) and siRNA-OCT1 (#1, #2, #3) against different sites in DLX6-AS1 and OCT1, and negative control siRNA (si-NC), were transfected into SGC7901 and AGS cells using the Lipofectamine 3000 Reagent (Invitrogen). MiR-204-5p inhibitor as well as miRNA negative control (miR-NC) purchased from GenePharma were transfected into cells by the same method.