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miR-133a represses tumour growth and metastasis in colorectal cancer by targeting LIM and SH3 protein 1 and inhibiting the MAPK pathway

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Abstract In recent studies of microRNA expression, miR-133a deregulation was identified in colorectal carcinoma (CRC). However, the mechanisms underlying the pathogenesis and progression of CRC are poorly understood. We found that miR-133a expression was usually down-regulated in CRC cell lines and tissue specimens. Ectopic miR-133a expression inhibited cell proliferation and cell migration. Stable overexpression of miR-133a was sufficient to suppress tumour growth and intrahepatic and pulmonary metastasis *in vivo*. Additional studies showed that miR-133a can target the 3′ untranslated region (3′UTR) of *LIM and SH3 protein 1* (*LASP1*) mRNA and suppress the expression of LASP1, which we identified in previous studies as a CRC-associated protein. In contrast to the phenotypes induced by miR-133a restoration, LASP1-induced cell proliferation and migration rescued miR-133a-mediated biological behaviours, as did LASP1 overexpression. Investigations of possible mechanisms underlying these behaviours revealed that miR-133a modulates the expression of key cellular molecules and participates in the MAPK pathway by inhibiting phosphorylation of ERK and MEK. miR-133a may play a key role in CRC genesis and metastasis, which suggests its potential role in the molecular therapy of cancer.

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1. Introduction

Colorectal cancer (CRC) is the second leading cause of death from cancer worldwide. In China, CRC ranks fifth among cancer deaths and its incidence is continually increasing [1]. Despite advancements made in surgical techniques, radiotherapy, and chemotherapy for patients with CRC over the last several decades, the overall survival rate has not improved markedly. Metastasis plays a critical role in the poor prognosis. Little is known about the exact mechanisms underlying the metastasis of CRC. Once key factors in cancer progression are identified, new diagnostic strategies and drugs targeting these markers of progression can be developed accordingly.

MicroRNAs (miRNAs) comprise a class of diverse, small, non-coding RNAs that function as critical gene regulators. Bioinformatic analyses indicate that each miRNA regulates hundreds of target genes, underscoring their potential influence on almost every biological pathway [2,3]. Recently, evidence has been provided showing that approximately half of all human miRNAs are located in cancer-associated genomic regions and can function as tumour suppressor genes or oncogenes, depending on their targets [4–6]. To date, several human miRNAs have been shown to be dysregulated in CRC—including miR-106a, miR-23a, miR-222, miR-17, miR-30a-5p and miR-34a [7–12]—and, thus, may contribute to the development and progression of CRC. These findings suggest the involvement of miRNAs in CRC tumourigenesis. A recent systematic review of profiling studies and experimental validation revealed that miR-133a is down-regulated in CRC tissue compared with adjacent normal tissue. This finding was determined by miRNA microarray and quantitative real-time polymerase chain reaction (qRT-PCR) analysis and was consistent across four studies [13]. The role of miR-133a during different stages of CRC progression appears to be controversial, however, because in addition to tumour suppression, it may enhance brain metastasis [14]. Until now, no evidence of a functional role for miR-133a in CRC has been documented.

In this study, we investigated the involvement of miR-133a in CRC by examining its expression in human CRC cells and tissue samples; its effects on cell growth, cell-cycle distribution and cell migration; and its role in CRC tumourigenesis and metastasis in a murine model while searching for mechanism(s) underlying its activity in CRC, all with the intended goal of improving our understanding of CRC development and progression.

2. Materials and methods

2.1. Cell culture and miRNA transfection

CRC cell lines HT29, HCT116, SW480 and SW620 were obtained from the American Type Culture Collection (ATCC; Manassas, VA) and maintained as previously described [15]. Additionally, a human CRC cell subline with unique liver metastatic potential, designated SW480/M5, was established in our laboratory [16] and used in the analysis.

The cells were cultured in RPMI 1640 (Hyclone; Logan, UT, United States of America (USA)) supplemented with 10% foetal bovine serum (FBS) (Gibco-BRL, Invitrogen; Paisley, United Kingdom (UK)) at a humidity of 5% CO_2 at 37 °C. miRNAs were transfected at a working concentration of 100 nmol/L using Lipofectamine 2000 reagent (Invitrogen; Carlsbad, CA, USA). The miR-133a mimic, a non-specific miR control, anti-miR-133a, and a non-specific anti-miR control were all purchased from GenePharma (Shanghai, China). Protein and RNA samples were extracted from subconfluent cells during the exponential phase of growth.

2.2. Tumour tissue sample

Fresh primary CRC specimens and paired non-cancerous colorectal tissue were provided by the Tumor Tissue Bank of Nanfang Hospital. In each case, a diagnosis of primary CRC had been made, and the patient had undergone elective surgery for CRC in Nanfang Hospital between 2007 and 2010. The pathological diagnosis was made in the Department of Pathology of Nanfang Hospital of Southern Medical University. The study was approved by the Ethics Committee of Southern Medical University and all aspects of the study comply with the Declaration of Helsinki. Ethics Committee of the Southern Medical University specifically approved that not informed consent was required because data were going to be analysed anonymously.

2.3. RNA isolation, reverse transcription and quantitative real-time PCR

Total RNA was extracted using Trizol reagent (Invitrogen). To quantitate miR-133a expression, total RNA was polyadenylated and underwent reverse transcription using an NCode miRNA First-Strand cDNA Synthesis kit (Invitrogen). mRNA levels in the LIM and SH3 protein 1 (LASP1) gene were measured as previously described [15]. qRT-PCR was carried out using an SYBR Green PCR master mix (Applied Biosystems; Foster City, CA, USA) on an ABI 7500HT system. *GAPDH* or *U6* snRNA was used as an endogenous control. All samples were normalised to internal controls, and fold changes were calculated through relative quantification ($2^{-\Delta\Delta CT}$).

2.4. Western blot analysis

Protein expression was assessed by immunoblot analysis of cell lysates (20–60 μg) in RIPA buffer in the