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MiR-103 regulates hepatocellular carcinoma growth by targeting AKAP12

Wei Xia[a,1], Jing Ni[a,1], Juhua Zhuang[a], Leixing Qian[a], Peng Wang[b,**], Jiening Wang[b,*]

[a] Department of Nuclear Medicine, Shanghai Seventh People's Hospital, Shanghai, China
[b] President's Office of Shanghai Seventh People's Hospital, Shanghai, China

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ABSTRACT

AKAP12/Gravin (A kinase anchor protein 12) belongs to the group of A-kinase scaffold proteins and functions as a tumor suppressor in some human primary cancers. While AKAP12 is found consistently downregulated in hepatocellular carcinoma (HCC), its involvement in hepatocarcinogenesis has not been fully elucidated. We identified targeting sites for miR-103 in the 3′-untranslated region (3′-UTR) of AKAP12 by bioinformatic analysis and confirm their function by a luciferase reporter gene assay. We reveal miR-103 expression to be inversely correlated with AKAP12 in HCC tissue samples and show that overexpressed miR-103 promotes cell proliferation and inhibits apoptosis by downregulating AKAP12 expression in HCC cell lines. On the other hand, repression of miR-103 suppresses proliferation and promotes apoptosis in HCC cells by increasing AKAP12. In xenografted HCC tumors, overexpression of AKAP12 suppresses tumor growth whereas overexpression of miR-103 enhances tumor growth while repressing AKAP12. Since the activation of telomerase is crucial for cells to gain immortality and proliferation ability, we investigated whether AKAP12 expression affected telomerase activity in HCC cells. Both AKAP12 overexpression and protein kinase Cα (PKCα) inhibition prevent nuclear translocation and phosphorylation of TERT and reduce telomerase activity in HCC cells. These findings indicate that miR-103 potentially acts as an oncogene in HCC by inhibiting AKAP12 expression and raise the possibility that miR-103 increases telomerase activity by increasing PKCα activity. Thus, miR-103 may represent a new potential diagnostic and therapeutic target for HCC treatment.

1. Introduction

Primary liver cancer (hepatocellular carcinoma, HCC) is the fifth most common cause of cancer in men, the seventh in women and the third most frequent cause of cancer death worldwide (Ferlay et al., 2010). The incidence of HCC has increased in recent decades because of the rising incidence of hepatitis C viral infection and other causes of cirrhosis (Jemal et al., 2011; Perz et al., 2006). Although surgical hepatic resection and liver transplantation techniques have improved, the overall 5-year survival rate is still only around 5% and the long-term prognosis remains discouraging (Fong and Tanabe, 2014). HCC is an epithelial tumor that can originate from mature hepatocytes or stem cells (van Malenstein et al., 2011), with hepatocarcinogenes being a multistep process involving different genetic alterations that ultimately lead to malignant transformation (De Minicis et al., 2013). A better understanding of the molecular pathways involved in the etiology and progression of HCC may lead to improved treatments.

Kinase scaffolds such as A-kinase scaffold proteins have become a recent focus in drug discovery because of their unique properties as therapeutic targets (Esseltine and Scott, 2013; Hoshi et al., 2010). AKAP12 is an A-kinase scaffold protein whose loss is associated with increased cancer susceptibility, including to HCC (Hayashi et al., 2012). AKAP12 is classified as a class II tumor suppressor gene based on its ability to suppress growth rates and promote reorganization of the actin-based cytoskeleton in v-Src-transformed fibroblasts (Lin et al., 1995; Nelson and Gelman, 1997). Our current molecular understanding of AKAP12 is that it serves as a scaffold protein in signal transduction, thereby coordinating signal pathways for cellular senescence and oncogenic progression

Abbreviations: HCC, hepatocellular carcinoma; AKAP12, A kinase anchor protein 12; TERT, telomerase reverse transcriptase; PK, protein kinase; UTR, untranslated region; ORF, open reading frame.

* Corresponding author. Tel.: +86 02150778729; fax: +86 02158678350.
** Corresponding author. Tel.: +86 02150778729; fax: +86 02158678350.
E-mail addresses: awingxia@foxmail.com (P. Wang), doctorwjn@126.com (J. Wang).

[1] These authors contributed equally to this work and should be considered co-first authors.

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(Gelman, 2012). While AKAP12 is a major protein kinase (PK) C substrate and binding protein whose expression is downregulated in Src- and Ras-transformed cells (Chapline et al., 1996; Lin et al., 1996), AKAP12 also binds key signaling mediators such as PKC, PKA, calmodulin, F-actin, cyclins, Src and phospholipids in a spatiotemporal manner (Gelman, 2010). Reduced AKAP12 expression has been consistently observed in HCC (Goeppert et al., 2010; Hayashi et al., 2012), suggestive of a tumor suppressor role. The molecular mechanism by which AKAP12 potentially exerts tumor suppressor function in HCC remains unexplored.

MicroRNAs (miRNAs) are a class of small, non-coding endogenous RNAs 21–30 nucleotides in length that, in the case of metazoans, regulate protein-coding genes by directing their translational repression, mRNA destabilization or a combination of the two (Bartel, 2009). miRNAs play important regulatory roles in many biological processes, including human carcinogenesis, and are therefore promising targets for tumor diagnosis and therapy (Ambros, 2004; Bartel, 2004).

In this study we show that AKAP12 overexpression suppresses HCC proliferation and promotes apoptosis in vitro and in HCC xenografts. We identify miR-103 as a potential repressor of AKAP12 and demonstrate its ability to promote HCC proliferation by directly targeting the 3′untranslated region (3′UTR) of AKAP12. We show that both forced AKAP12 expression as well as PKCα inhibition repress telomerase activity in HCC cells, suggesting that AKAP12's tumor suppressive function in HCC is potentially mediated by suppressing telomerase activity via PKCα inhibition. Our results indicate that miR-103 upregulation may contribute to HCC development and progression via repression of AKAP12, highlighting miR-103 as a potential target for HCC therapy.

2. Materials and methods (details in Supplemental information)

2.1. Cell culture

The HCC cell lines were purchased from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China).

2.2. Tissue specimens

This study was conducted on 12 pairs of snap-frozen HCC tumor tissues and matched normal tissues from adjacent regions, which were diagnosed histopathologically at Shanghai Seventh People's Hospital.

2.3. RNA isolation and quantitative RT-PCR

miRNAs were quantified using Taqman miRNA probes (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

2.4. Overexpression and knockdown of miR-103

Synthetic pre-miR-103, anti-miR-103 and scrambled negative control RNAs (pre-scramble and anti-scramble) were purchased from Ambion (Austin, TX, USA).

2.5. Luciferase reporter assay

A luciferase reporter assay was performed as previously described (Chen et al., 2009) to test the direct binding of miR-103 to the target gene AKAP12.

2.6. Plasmid construction and siRNA interference assay

The siRNA sequence targeting the human AKAP12 cDNA was designed and synthesized by GenePharma (Shanghai, China).

2.7. miR-103 lentiviral vector

Lentivirus for miR-103 overexpression was purchased from Invitrogen.

2.8. Protein extraction and western blotting

The protein levels were analyzed by western blot using the corresponding antibodies.

2.9. Cell viability assay

MTT assay (Sigma-Aldrich, St Louis, MO, USA) was performed according to the manufacturer's instructions.

2.10. Apoptosis assays

Apoptosis was determined using an Annexin V-FITC/propidium iodide (PI) staining assay.

2.11. PKCα activity assay

PKCα kinase activity was measured using the PepTagR Nonradioactive Protein Kinase Assays (PromegaCorp, Madison, WI, USA).

2.12. Preparation of nuclear extracts, immunoprecipitation and immunoblot

Immunoprecipitation was performed as described previously (Jagadeesh et al., 2006).

2.13. Telomeric repeat amplification protocol (TRAP) assay

The TRAP assay was performed on cell extracts as previously described (Jagadeesh et al., 2006).

2.14. Tumor xenografts in mice

All experimental procedures were approved by the Ethics Committee of Seventh Peoples Hospital of Shanghai.

2.15. Statistical analysis

The data shown are the mean ± SE of at least three independent experiments. The differences were considered statistically significant at $P < 0.05$ using the Student's t-test or one-way repeated measures ANOVA.

3. Results

3.1. AKAP12 mRNA and protein are downregulated in hepatocellular carcinoma tissues

We determined the expression levels of AKAP12 mRNA (isoform α) and protein in 12 HCC tissue samples by qPCR and western blotting, respectively. AKAP 12 protein levels were significantly lower in all 12 samples compared to their normal adjacent tissue pairs ($P < 0.01$; Fig. 1A and B) confirming observations made previously (Goeppert et al., 2010; Hayashi et al., 2012). In ten out of the 12 samples, AKAP12 mRNA levels were significantly lower compared to their normal adjacent tissue pairs