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MiR-509-3-5p causes aberrant mitosis and anti-proliferative effect by suppression of PLK1 in human lung cancer A549 cells

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ABSTRACT

MicroRNAs (miRNAs) are potent post-transcriptional regulators of gene expression and play roles in DNA damage response (DDR). PLK1 is identified as a modulator of DNA damage checkpoint. Although down-regulation of PLK1 by certain microRNAs has been reported, little is known about the interplay between PLK1 and miR-509-3-5p in DDR. Here we have demonstrated that miR-509-3-5p repressed PLK1 expression by targeting PLK1 3′-UTR, thereby causing mitotic aberration and growth arrest of human lung cancer A549 cells. Repression of PLK1 by miR-509-3-5p was further evidenced by over-expression of miR-509-3-5p in A549, HepG2 and HCT116p53$^{-/-}$ cancer cells, in which PLK1 protein was suppressed. Consistently, miR-509-3-5p was stimulated, while PLK1 protein was down-regulated in A549 cells exposed to CIS and ADR, suggesting that suppression of PLK1 by miR-509-3-5p is a component of CIS/ADR-induced DDR pathway. Flow cytometry and immunofluorescence labeling showed that over-expression of miR-509-3-5p in A549 induced G2/M arrest and aberrant mitosis characterized by abnormal bipolar mitotic spindles, condensed chromosomes, lagging DNA and chromosome bridges. In addition, over-expression of miR-509-3-5p markedly blocked A549 cell proliferation and sensitized the cells to CIS and ADR treatment. Taken together, miR-509-3-5p is a feasible suppressor for cancer by targeting PLK1. Our data may provide aid in potential design of combined chemotherapy and in our better understanding of the roles of microRNAs in response to DNA damage.

1. Introduction

MicroRNAs (miRNAs) target well around one third of mammalian genes, regulating diverse cellular processes [1]. MicroRNAs may act as oncogene and/or suppressor in cancer cells and tissues [2,3], and many of them are found to modulate cell cycle progression and induce apoptosis in DNA damage response (DDR) [4]. To illustrate, up-regulation of miR-34a by p53 induces G1 arrest and apoptosis, acting as a tumor suppressor [5–7]. Over-expressed miR-182 acts as an oncogene in p53-deficient cells [8]. Besides, several microRNAs have been found to promote anticancer effects of DNA damage-based radiotherapy and chemotherapy. For instance, miR-521 modulates radiosensitivity of prostate cancer cells by suppressing DNA repair protein Cockayne syndrome protein A [9]. Suppression of H2AX by miR-24 enhances sensitivity of hematopoietic cells to irradiation and genotoxic drugs by reducing DNA repair capacity of the cells [10].

MiR-509-3-5p is a member of miR-506 family consisting of miR-506, miR-508-3p, miR-509-3p, miR-509-5p, miR-509-3-5p, miR-510 and miR-514. They are tandem clustered in the same genomic region (Xq27.3) and co-expressed in several cancer cells, including epithelial ovarian cancer (EOC) and clear cell renal cell carcinoma (ccRCC) cells [11,12]. MiR-506 is detected to be the most significant miRNA in regulating gene expression in prostate cancer cells [13], and elevated miR-506 may sensitize ovarian cancer cells to platinum-induced DNA damage by targeting DNA damage repair gene RAD51 [14]. Overexpression of miR-508-3p and miR-509-3p suppress RCC cell proliferation and migration *in vitro* [15].

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Likewise, miR-509-3p functions as a tumor suppressor in hepatoma cells [16]. However, the function(s) of miR-509-3-5p has not been identified, and little is known about its roles in chemotherapeutic drug-induced DDR.

Polo-like kinase 1 (PLK1) is highly expressed in a variety of cancers, thus became a critical target for anticancer therapy. PLK1 maintains genomic stability by modulating error-free DNA replication and accurate chromosome segregation in DNA damage checkpoint [17–20]. Stimulating PLK1 expression promotes tumorigenesis by inducing chromosome instability and aneuploidy, while silencing PLK1 blocks tumor growth *in vitro and in vivo* [21,22]. Recent studies show that several miRNAs including miR-210, miR-10b*, miR-100, miR-593*, and miR494, which target PLK1, block tumor growth and progression [23–27]. Here we describe a novel regulatory mechanism of PLK1 gene, in which miR-509-3-5p represses PLK1 expression by targeting PLK1 3′-UTR. Down-regulation of PLK1 by miR-509-3-5p causes mitotic aberration and G2/M arrest in human lung cancer A549 cells. Moreover, over-expression of miR-509-3-5p sensitizes A549 cells to cisplatin (CIS) and adriamycin (ADR). These data may provide evidence for potential design of the combined therapy of chemotherapeutic drugs with microRNAs.

2. Materials and methods

2.1. Cell culture and drug treatment

Human lung cancer A549 cells (ATCC, Manassas, VA, USA), as well as HepG2 and HCT116p53$^{-/-}$ cells, were cultured in DMEM medium supplemented with 10% fetal bovine serum (GIBICO, BRL), 100 U/ml penicillin and 100 mg/ml streptomycin, and grown at 37 °C with 5% CO_2. Cells were exposed to cisplatin (CIS) or adriamycin (ADR) for given hours.

2.2. MiRNAs, siRNAs and transfection

MiR-509-3-5p mimic, inhibitor, scrambled RNAs, and PLK1 siRNA duplexes were from GenePharma (Shanghai, China). Transfection was performed with Lipofectamine 2000 (Invitrogen) following manufacturer's protocol. The PLK1 siRNA sequence was 5′-GGGCGGCUUUGCCAAGUGCTT-3' [28]. The sequence of miR509-3-5p was 5′-UACUGCAGACGUGGCAAUCAUG-3'.

2.3. RNA isolation and real-time quantitative PCR (RT-qPCR)

Total RNAs were extracted using Trizol (Invitrogen) according to the manufacturer's instructions. For mRNA detection, 20 μg total RNAs were subjected to reverse transcription using Reverse Transcription Kit (Thermo Scientific, Waltham, MA USA); for analyzing mature miRNAs, 2 μg total RNAs was reverse transcribed using Reverse Transcription Kit. Stem-loop RT primers for mature miRNAs and oligo (dT) were used for reverse transcription. PCR amplification was performed with the primers as follows: For GAPDH, (forward) 5′-TGTCAGTGGTGGACCTGACCT-3′ and (reverse) 5′-AGGGGAGATTCAGTGTGGTG-3'; For PLK1, 5′-GGCAACCTTTTCCTGAATGA-3′ and 5′-AATGGACCACACATCCACCT-3'; For miR-509-3-5p, 5′-ACACTCCAGCTGGGTACTGCAGACGTGGCA-3′ and 5′-TGGTGTCGTGGAGTCG-3'; Stem-loop RT primer for miR-509-3-5p, 5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCATGATTG-3.
Each PCR mixture contained 2 μl cDNA, 400 nM of each primer and 12.5 μl of the Power SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA) in a 25 μl reaction mixture. RT-qPCR was performed using ABIPRISM 7500 Real-Time PCR system (Applied Biosystems, Foster City). RT-qPCR data were normalized to GAPDH or U6 according to the manufacturer's protocol.

2.4. Western blotting

Whole-cell extracts were prepared and protein concentration was quantified. Twenty micrograms of total proteins were subjected to 10% SDS–PAGE, transferred onto nitrocellulose membranes, and probed with specific antibodies against PLK1 (abB17056, Abcam) or β-actin (PM-053, MBL). After incubated overnight, proteins on the membrane were immunostained with a IRDye 800CW secondary antibody (IgG) for 1 h at room temperature. Blots were visualized with LI-COR Odyssey image analysis system (Li-cor Biosciences, Cambridge, UK).

2.5. Luciferase reporter constructs and luciferase assay

For construction of luciferase reporter plasmids containing PLK1 3′-UTR and the binding site mutant of miR-509-3-5p, the sequences of PLK1 3′-UTR (+1866/+2170) and its mutant (Fig. 1B) were obtained by PCR and inserted into the pMIR-Report plasmid (Applied Biosystems) at the *Spe* I and *Hin*d III sites to generate pMIR-Report-PLK1-3′-UTR-WT (wild type) and pMIR-Report-PLK1-3′-UTR-MT (mutant). For reporter enzyme assays, cells were co-transfected with 100 ng firefly luciferase reporter wild-type or mutant vector, 5 ng renilla luciferase (for normalizing transfection efficiency) and 10 nM miR-509-3-5p mimics. After 48 h transfection, luciferase

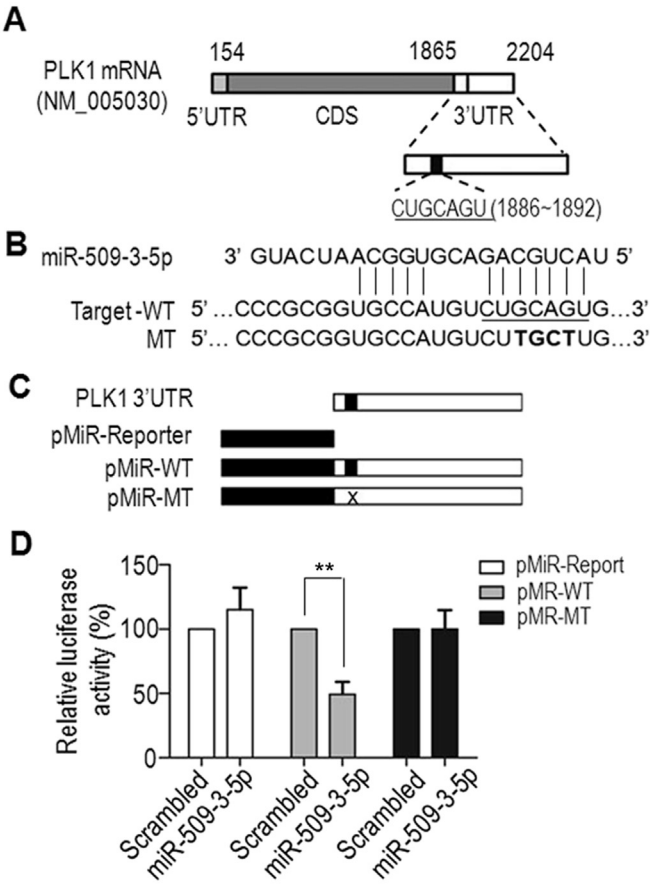

Fig. 1. MiR-509-3-5p down-regulates PLK1 expression by targeting PLK1 3′-UTR. (**A**) The miR-509-3-5p binding site (underlined) in PLK1 3′-UTR. (**B**) The predicted miR-509-3-5p binding sequence and mutated version of PLK1 3′-UTR. WT/wild type; MT/mutant (mutated bases are boldface). (**C**) Interpretation of the luciferase reporter plasmids containing full-length PLK1 3′-UTR or mutant. (**D**) Relative luciferase activities of the reporter plasmids in A549 cells. The activity of scrambled RNA transfection is normalized to "1" (i.e., 100%). Data present mean ± S.D. (n = 3). $^{**}p = 0.0062$.