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5′-Triphosphate siRNA targeting *MDR1* reverses multi-drug resistance and activates RIG-I-induced immune-stimulatory and apoptotic effects against human myeloid leukaemia cells

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ABSTRACT

Multi-drug resistance (MDR), immune suppression and decreased apoptosis are important causes of therapy-failure in leukaemia. Short interfering RNAs (siRNAs) down-regulate gene transcription, have sequence-independent immune-stimulatory effects and synergize with other anti-cancer therapies in some experimental models. We designed a siRNA targeting *MDR1* with 5′-triphosphate ends (3p-siRNA-MDR1). Treatment of leukaemia cells with 3p-siRNA-MDR1 down-regulated *MDR1* expression, reduced-drug resistance and induced immune and pro-apoptotic effects in drug-resistant HL-60/Adr and K562/Adr human leukaemia cell lines. We show mechanisms-of-action of these effects involve alterations in the anti-viral cytosolic retinoic acid-inducible protein-I (RIG-I; encoded by *RIG-I* or *DDX58*) mediated type-I interferon signal induction, interferon-gamma-inducible protein 10 (IP-10; encoded by *IP10* or *CXCL10*) secretion, major histocompatibility complex-I expression (MHC-I) and caspase-mediated cell apoptosis. 3p-siRNA-MDR1 transfection also enhanced the anti-leukaemia efficacy of doxorubicin. These data suggest a possible synergistic role for 3p-siRNA-MDR1 in anti-leukaemia therapy.

1. Introduction

Most persons with acute myeloid leukaemia fail therapy. Development of multi-drug resistance (MDR) is thought to be an important cause. MDR is characterized by cross-resistance to several anti-cancer drugs with diverse chemical structures and mechanisms-of-action [1]. MDR involves the expression and activity of an ATP-binding-cassette (ABC; CD243) transporter also known as P-glycoprotein (P-gp), an efflux pump protein which extrudes anti-cancer drugs from cells [2]. Additionally, P-gp is associated with decreased caspase activation resulting from altered activity of several chloride channels [3,4] altered intra-cellular pH [5] and elevated levels of B-cell lymphoma protein-2 (Bcl-2) [6,7]. P-gp also has anti-apoptotic effects on cancer cell lines including the K562 human leukaemia cell line [8,9].

Considerable data indicate down-regulating *MDR1* by RNA interference (RNAi) decreases levels of P-gp expression which can reverse MDR in leukaemia cells [10–12]. Treatment of human cells and cell lines with short interfering (siRNA) and short hairpin RNAs (shRNA) induces production of type-I interferons (IFN-α and -β) and induces immune stimulatory cytokine secretion, an effect which is RNA- sequence independent [13]. Specifically, the 5′-triphosphate group at the end of virus RNAs and siRNAs synthesized from the T7 RNA polymerase system is detected by retinoic acid-inducible protein I (RIG-I encoded by *RIG-I* or *DDX58*), a ubiquitously expressed cytosolic pattern recognition receptor responsible for innate anti-viral immune responses [14,15]. A 5′-triphosphate siRNA targeting *BCL2* (3p-siRNA-BCL2) up-regulated major histocompatibility complex class-I (MHC-I) expression and the caspase-mediated intrinsic mitochondrial apoptotic pathway in human melanoma cells. The anti-cancer efficacy of 3p-siRNA-BCL2 was more potent than siRNA with only a *BCL2*-silencing effect [16]. In addition, 5′-triphosphate siRNA had greater efficacy in other human solid cancer models and cell lines including pancreas [17], lung [18,19], ovary [20] and brain cancers [21].

A recent study reported anthracyclines, widely used in leukaemia therapy, can mimic viruses by inducing immune responses including IFN-I production and triggering release of IP10,

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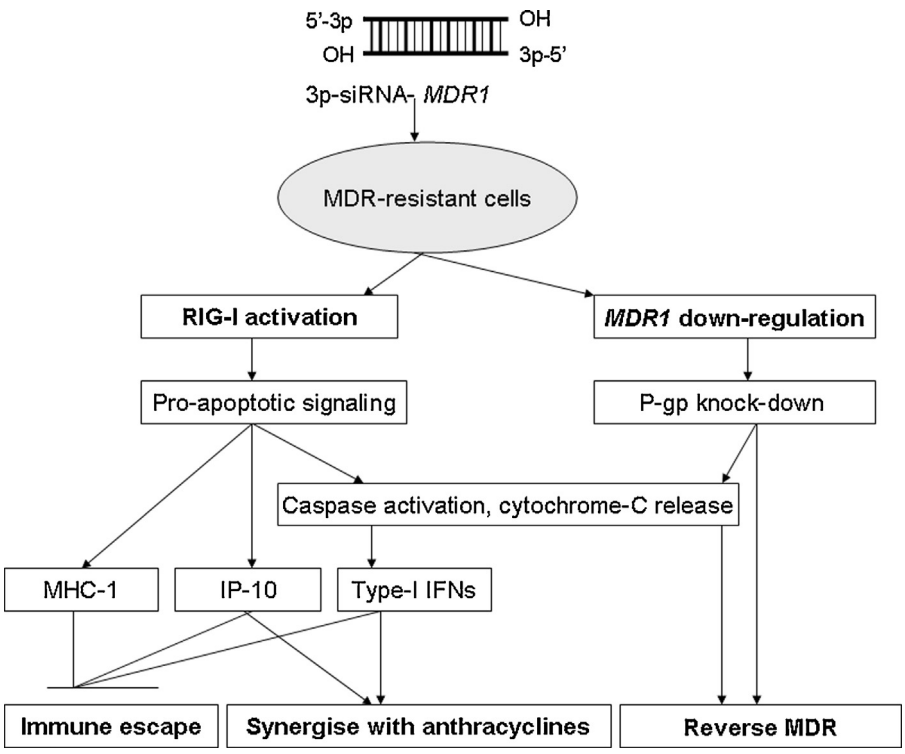

Fig. 1. Mechanisms of 3p-siRNA-MDR1.

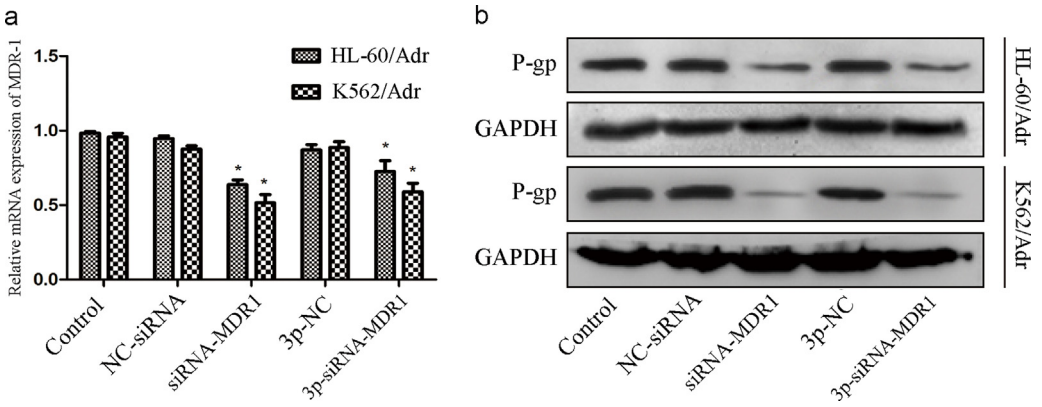

Fig. 2. 3p-siRNA-MDR1 silences P-gp expression in HL-60/Adr and K562/Adr cells. (a) Relative MDR1 mRNA level was quantified by qRT-PCR in HL-60/Adr and K562/Adr cells 24 h after transfection with NC-siRNA, siRNA-MDR1, 3p-NC, or 3p-siRNA-MDR1 (1 μg/ml). (b) Western blot analysis of P-gp protein level in HL-60/Adr and K562/Adr cells 48 h after transfection with RNAs as indicated. Data are presented as Mean ± SD (n = 3). *P < 0.05 vs. control.

molecules which induce drug-sensitivity in some drug-resistant cancers [22]. Our aim was to construct a multi-functional 5′-triphosphate modified *MDR1* specific siRNA (3p-siRNA-MDR1) able to reverse MDR, induce innate immunity and synergize with doxorubicin. We used the human multi-drug resistant leukaemia cell lines HL-60/Adr and K562/Adr as models. Relationships between theses complementary mechanisms of leukaemia control are shown in Fig. 1.

2. Materials and methods

2.1. Cell culture, antibodies and reagents

The human drug-resistant leukaemia cell line HL-60/Adr and K562/Adr were provided by KeyGen Biotech Co., Ltd. (Nanjing, China) and cultured in RPMI-1640 medium (Gibco Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Sijiqing Biotech, Hangzhou, China), and 1% penicillin-streptomycin (KeyGen Biotech). Cells were maintained at 37°C in a 5% CO_2 humidified atmosphere. Doxorubicin (1 μg/ml) (Hisun Pharmaceutical Co. Ltd., Zhejiang, China) was added to the medium to maintain drug resistance.

2.2. siRNAs and transfection

siRNA to *MDR1* and *RIG-I* were synthesized by GenePharma (Shanghai, China). The 5′-triphosphate-modified siRNA against *MDR1* (3p-siRNA-MDR1) was transcribed using the correlating DNA template that contained the T7 RNA polymerase promoter sequence. In vitro transcription and purification of 3p-siRNA-MDR1 was done using the AmpliScribe T7-Flash Transcription Kit (Epicenter Biotechnologies, Madison, WI, USA) according to the manufacturer's instruction. MDR1 silencing siRNA (siRNA-MDR1 and 3p-siRNA-MDR1) sequences