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Inhibitory effect of siRNA targeting IGF-1R on endometrial carcinoma

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ABSTRACT

The type-1 insulin-like growth factor receptor (IGF-1R) is one member of tyrosine protein kinase receptor family. It is a causal factor for tumor initiation, development and frequently overactivated in a variety of human malignancies, including endometrial carcinoma. To investigate its possibility as a therapeutic target for endometrial carcinoma, we adopted RNA interference technology to down-regulate IGF-1R expression in endometrial carcinoma and analyzed its apoptosis inductive effect and tumorigenicity in vivo. Results showed that RNAi mediated down-regulation of IGF-1R expression in endometrial carcinoma significantly induced apoptosis, reduced downstream protein phosphorylation and decreased tumorigenicity in vivo accompanied with lower proliferation index in tumor tissue, Which implied the therapeutic potential of RNAi in the treatment of endometrial carcinoma by targeting IGF-1R and IGF-1R may be a potential therapeutic target for human endometrial carcinoma.

1. Introduction

Endometrial carcinoma is one of the most malignancies in female genital tract and the third most common cause of gynecologic cancer death. The incidence and mortality rates of endometrial carcinomas have increased in recent years with 40100 new cases and 7470 deaths reported in 2008 [1]. This emphasizes the need for a detailed understanding of the molecular basis of endometrial carcinogenesis, which may lead to better diagnostic and therapeutic interventions for the disease.

Many genetic and epigenetic agents are involved in tumorigenesis and progression [2]. Endometrial carcinoma possesses the capability of using normal or oversecreted extracellular signal for proliferation and/or anti-apoptosis to create a growth priority over normal cells. The major players in extracellular signal are growth factor receptors. The type-1 insulin-like growth factor receptor (IGF-1R) is one member of tyrosine protein kinase receptor family, which is important for the establishment of a malignant cell phenotype [3], cell metastasis [4], protection from apoptosis [5] and enhancement of cell proliferation [6]. In human endometrial carcinoma, levels of IGF-1R have been correlated with tumor progression. High IGF-1R expression in endometrial carcinoma has also been found to be an important prognostic factor [7]. In addition, endometrial carcinoma cells can synthesize and secrete IGF-I and IGF-II, which can integrate with their IGF-1R membrane receptor and cause continuous proliferation of the tumor cells [8].

Although many researches suggest that IGF-1R play a pivotal role in endometrial carcinogenesis, few works indicate IGF-1R can be used as the target for molecular targeting therapy and how the receptor can be targeted. Here we focus on these two problems. Despite various IGF-1R targeting approaches have so far been developed, such as monoclonal antibodies [9,10], small molecular tyrosine inhibitor [9,11] and antisense oligonucleotides [9,12]. But their homology with insulin receptor and low efficiency have limited their applications. RNA interference (RNAi) is a post transcriptional gene silencing mechanism, which has provided new opportunities for experimental biology [13–15], and demonstrated great prospects for specific gene function, signal transduction and gene therapy. Many vectors such as plasmid, adenovirus, retrovirus [16] have been developed to carry small interference RNA (siRNA), but their low efficiency and short half-life hamper further application. Lentivirus is a genus of slow viruses of the retroviridae family, which is characterized by a long incubation period, minimal immunogenicity [17] and can deliver a significant amount of genetic information into the DNA of the host cell, resulting in stable transcript knockdown and a high efficiency RNAi delivery. Although lentivirus has been used as gene delivery vectors for many years, the use of lentiviral vector expressing RNAi as a therapeutic tool for endometrial carcinoma has not been clearly investigated.

In this study, we employed the highly efficient lentivirus vector to deliver and express the siRNA targeting IGF-1R to determine whether this technique could be used for the specific inhibition of IGF-1R overexpression and whether this inhibition resulted in anti-tumor effects in hope of providing a new method to conquer endometrial carcinoma.

2. Materials and methods

2.1. Endometrial carcinoma cell line

Human endometrial carcinoma cell lines HEC-1A and HEC-1B were provided by American Type Culture Collection (Manassas,USA). The

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cells were routinely cultured in Dulbecco's modification of Eagle's medium (Gibco,USA) supplied with 10% fetal calf serum (Hyclone, USA), penicillin (100 U/ml) and streptomycin (0.1 mg/ml), at 37 °C in the humidified atmosphere of a 5% CO_2 incubation.

2.2. Construction and transfection of the siRNA lentivirus expression vector

Five RNAi candidate target sequences to human IGF-1R (GenBank: NM_000875) were designed following the procedure of Dharmacon siDESIGN center, and were cloned into pGCL-GFP vector. The one (the targeting sequence was 5'-GCC GAT GTG TGA GA AGC-3') had the best interference efficiency in 293 T cells cotransfected with IGF-1R and siRNA expression constructs revealed by western blot and was selected to knockdown the endogenous IGF-1R in endometrial carcinoma cells. The lentivirus vector only expressing GFP was used as a negative control. The oligonucleotides encoding the IGF-1R-Si sequence and a loop sequence separating the complementary domains were synthesized and inserted into the pGCL-GFP (Genechem, Shanghai). The recombinant virus was packaged using Lentiviral Vector Expression Systems (Genechem, Shanghai,). The titer of lentiviruses was determined by serial dilution and counting transfected cell under a fluorescent microscopy. Cells were infected by enhanced infection solution and cultured in DMEM medium containing 10% FBS. The cells transfected with the IGF-1R-Si lentivirus were named KD, those transfected with the control lentivirus were named NC, and no transfection were named CON.

2.3. Real-time PCR for determination of IGF-1R mRNA

Total RNA was isolated using TRIzol reagent (Invitrogen,USA). The reverse transcription reaction was performed using the cDNA synthesis kit (Fermentas,USA). The following reaction was carried out with Real-time PCR System (Takara, Japan). The prime for IGF-1R is the forward:5'-TGC GTG AGA GGA TTG AGT TTC-3'(269 bp), the reverse: 5'-CTT ATT GGC GTT GAG GTA TGC-3'. The prime for the internal control gene β-actin is the forward: 5'-GGC GGC ACCA CCA TGT ACC CT-3'(202), the reverse:5'-AGG GGC CGG ACT CGT ATA CT-3'. The thermal cycle conditions were 95 °C for 15 s, 45 cycle of 95 °C for 5 s, 60 °C for 30 s. After the PCR reaction, the melting curve from 55 °C to 95 °C was read every 0.5 °C, holding for 4 s, then incubated at 95 °C for 60 s. Cycling conditions for β-actin were the same. The comparative Ct method was used to calculate the relative changes in gene.

2.4. Western blot analysis for expression of IGF-1R and downstream protein Akt

Western blot analysis was performed following standard methods. Cell lysates were separated by 12% SDS-polyacrylamide gel at a concentration of 30 μg protein of each sample per lane. Polyvinylidene difluoride membranes (Millipore, USA) were incubated overnight at 4 °C with primary polyclonal IGF-1R antibody (Cell Signal, USA, diluted 1:1000), total or phosphorylated Akt antibody (Cell Signal, USA, diluted 1:1000), then incubation with a peroxidase conjugated secondary antibody (Santa Cruze, USA, diluted 1:2000) for 1 h at room temperature. The immunodetection was performed according to

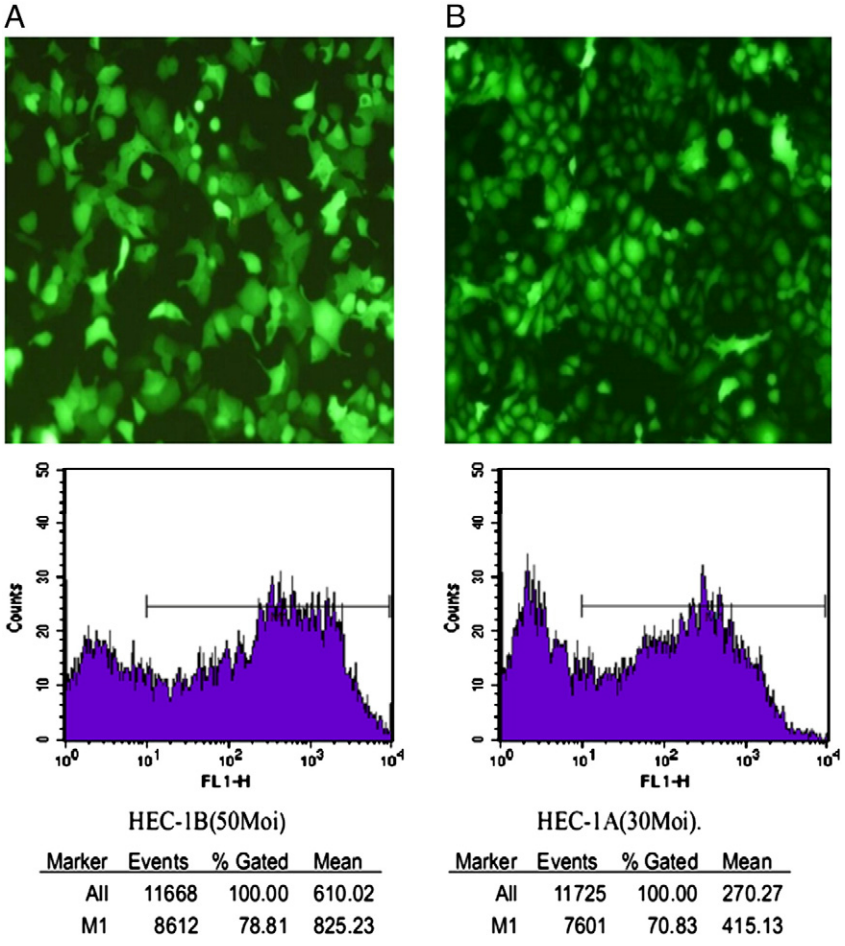

Fig. 1. Fluorescent micrographs and flow cytometry analysis transfection efficiency of cells transfected using lentivirus vector expressing green fluorescent protein after 72 h transfection. (original magnification × 200). High intensity of the fluorescence image indicated high efficiency of the transfection. (A) HEC-1B cells and transfection efficiency analysis. (B) HEC-1A cell and transfection efficiency analysis.