Knockdown of mediator complex subunit 19 inhibits the growth of ovarian cancer

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Received April 30, 2012; Accepted August 24, 2012

DOI: 10.3892/mmr.2012.1065

Abstract. Ovarian cancer causes more deaths than any other type of female reproductive cancer. The development of new therapeutic approaches is required due to the low survival rate using routine methods. The goal of this study was to investigate the effect of the gene silencing of mediator complex subunit 19 (MED19) on cell viability and tumor growth in ovarian cancer. Immunohistochemistry was used to characterize the expression of MED19 in human ovarian cancer tissues. Lentivirus-mediated RNAi was employed to downregulate endogenous MED19 expression in SKOV-3 and HEY ovarian cancer cells. MTT assay, BrdU incorporation assay, colony formation assay, cell cycle analysis and tumor xenografts in nude mice were performed to determine the effects of MED19 silencing on cell viability and tumor growth in vitro and in vivo. The data showed that the expression of MED19 in human ovarian cancer tissues correlated with the level of tumor malignancy. The downregulation of MED19 in ovarian cancer cells significantly inhibited cell proliferation and colony formation in vitro and led to cell cycle arrest in the G0/G1 phase. MED19 RNAi significantly inhibited ovarian cancer tumor growth in engrafted nude mice. Our findings reveal that the knockdown of MED19 by lentivirus-mediated RNAi may be useful in the treatment of human ovarian cancer.

Introduction

The management of ovarian cancer is a major challenge for gynecological oncologists. Since the symptoms of ovarian cancer are non-specific, more than 2/3 of the cases are diagnosed at advanced stages (1,2). Debunking surgery followed by chemotherapy continues to be the standard treatment for advanced ovarian cancer (3). Despite a high response to the initial therapy, many of the patients relapse eventually and succumb to chemotherapy-resistant diseases. As a result, the overall 5-year survival rate of advanced ovarian cancer remains low, at approximately 30% (1,4). The poor outcome of ovarian cancer necessitates our efforts towards better understanding its biological behavior and identifying new prognostic and therapeutic targets.

The mediator complex subunit 19 (MED19) was originally recognized in a search for mutants with increased aerobic expression of the CYC7 gene (5,6). The mediator complex is a multiprotein transcriptional co-activator that is expressed ubiquitously in eukaryotes and is required for the induction of RNA polymerase II transcription (7-9). A series of mediator complexes has been identified in mammals by multidimensional protein identification technology. These complexes include the thyroid hormone receptor-associated protein/SRB-Med-containing co-factor (TRAP/SMCC), the activator-recruited factor-large (ARC-L), vitamin D receptor-interacting protein (DRIP), mouse mediator, positive co-factor 2 (PC2) and the co-factor required for Sp1 transcriptional activation (CRSP) complexes (10).

The aberrant transcription of genes is believed to be one of the causes of human cancer. The silencing of MED19 may interfere with the transcriptional process and has been demonstrated to inhibit the growth of pancreatic cancer (11). The role of MED19 in ovarian cancer, however, has never been reported. The goal of this study was to investigate the effect of MED19 gene silencing on cell viability and tumor growth in ovarian cancer. Our results demonstrated that MED19 expression is correlated with malignancy and histological grading of human ovarian tumors. The knockdown of MED19 with lentivirus-mediated RNAi reduced the viability of ovarian cancer cells in vitro and inhibited tumor growth in xenografted nude mice. These data suggest that MED19 may serve as a potential target in the treatment of ovarian cancer.

Materials and methods

Cell lines. The SKOV-3 and HEY human ovarian cancer cell lines were purchased from ATCC (Manassas, VA, USA). The cells were maintained in RPMI-1640 medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS),
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100 U/ml penicillin and 100 µg/ml streptomycin and were cultured in a humidified atmosphere of 5% CO₂ at 37°C.

Tissue microarray and immunohistochemistry. Tissue microarrays of ovarian disease (OV1005) were obtained from Alenabio (Xi’an, China), and contained 18 ovarian cystadenoma, 7 varian borderline cystadenoma and 52 ovarian carcinoma tissue cores with 24 stage I, 9 stage II, 18 stage III and 1 stage IV ovarian cancer samples. Tissue microarray sections were stained with rabbit anti-MED19 antibody (1:250, Abcam Inc., Cambridge, MA, USA) using a goat anti-rabbit ABC immunohistochemistry kit (Mingrui Inc., Shanghai, China). The immunostaining patterns for MED19 were assessed by Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA).

Construction of lentiviral vectors. To generate lentivirus expressing RNAi specific for the MED19 gene, the RNA interference sequence for human MED19 (aaGGTGAGGAGA AGCTAAGT) was designed with the manufacturer's RNAi Designer program, and the negative control construct was created with a scrambled sequence (TTTCCGAACTGTC ACGT). The segments of nucleotides were cloned into the Hpa1 and XhoI sites of the pGCSIL-GFP vector (GeneChem, Shanghai, China) to generate pGCSIL-GFP-MED19 (siMed) and pGCSIL-GFP-nonsense (siNon).

Lentivirus transduction. Cells were plated at 40-50% confluence and incubated at 37°C. After 24 h of incubation, cells were infected with recombinant lentiviral vectors at a multiplicity of infection of 40 and then incubated at 37°C for 10 h. The viral supernatant was then replaced with fresh medium. At day 5 post-transduction, the knockdown efficiency of RNAi for MED19 were assessed by Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA).

RNA extraction and quantitative real-time RT-PCR. Total RNA was extracted from SKOV-3 cells with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The RNA expression levels of MED19 were detected by quantitative real-time RT-PCR and western blot analysis.

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Cell cycle analysis by flow cytometry. Cellular DNA content was detected with a flow cytometer (BD, Franklin Lakes, NJ, USA), in which 5x10⁴ events were collected. The list mode data were regrouped into DNA histograms, and individual cell cycle phase fractions were quantified using ModFit 3.0 analysis software (Verity Software Inc., Topsham, ME, USA).

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Tumor xenografts in nude mice. Female BALB/c mice were treated according to the protocol approved by the Ethics Committee of Fudan University. Six-week-old mice (n=4 per group) were injected subcutaneously either with parent SKOV-3...
cells, siNon-transduced SKOV-3 cells or siMed-transduced SKOV-3 cells (1x10^7 cells in 200 µl RPMI-1640) in the right forelimbs. The mice were sacrificed at day 42 after injection. Tumor size was measured with calipers weekly and tumor volume was calculated using the following formula: tumor volume (mm^3) = tumor length (mm) x tumor width (mm)/2. Bodyweight was also measured to assess any side-effects.

**Statistical analysis.** Immunohistochemistry data were analyzed by one-way ANOVA using SPSS software (Release 15.0, SPSS Inc.). Data from the in vitro studies were expressed as the means ± SE. The difference between the 2 groups was analyzed by one-way ANOVA followed by Dunnett's test. P<0.05 was considered to indicate a statistically significant difference. Representative images from microscopy and western blot analysis are shown.

## Results

### Expression levels of MEDI9 in benign, borderline and malignant ovarian tumor tissue.

MEDI9 expression in ovarian tumor tissue was examined by immunohistochemistry in a tissue microarray that contained ovarian cystadenoma, borderline cystadenoma and carcinoma samples. Following the incubation of the tissue microarrays with anti-MEDI9 antibody, the staining was mainly confined to the tumor tissue, whereas the majority of the mesenchymal tissue was unstained (Fig. 1A). The mean MEDI9 expression was significantly lower in the benign (0.008±0.009) or borderline ovarian cystadenomas (0.006±0.006) compared to the ovarian carcinomas (0.054±0.030) (P<0.01) (Fig. 1B). Among the ovarian carcinoma tissues, MEDI9 expression was higher in stage II and III than in stage I tumors (P<0.05). MEDI9 expression in the ovarian carcinoma tissues, however, did not correlate with age, tumor stage or histological type (P>0.05) (Table I).

### Knockdown of MEDI9 with lentiviral transduction in ovarian cancer cells.

The SKOV-3 and HEY human ovarian cancer cells were infected with lentivirus that contained either siMed or siNon. Efficient transduction was confirmed by GFP expression in cells observed under a fluorescent microscope (Fig. 2A). Twenty-four hours after transduction, the MEDI9 mRNA expression was downregulated by 84% in the cells transduced with siMed compared to those transduced with siNon (P<0.01) (Fig. 2B). A similar result was noted in western blot analysis which showed that MEDI9 protein expression was downregulated in the SKOV-3 cells following siMed transduction (Fig. 2C).

### Knockdown of MEDI9 inhibits the proliferation of ovarian cancer cells.

The proliferation dynamics of the parental, siNon-transduced and siMed-transduced cells were determined by MTT assays. Cell proliferation was monitored for...
The results showed that the growth of SKOV-3 and HEY cells transduced with siMed was significantly inhibited compared with the siNon-transduced and parental cells (Fig. 3A and B). A 70% reduction in viability was noted in the SKOV-3 cells with MED19 gene silencing compared with the siNon group on day 5.

To further investigate the effects of MED19 gene silencing on DNA synthesis in ovarian cancer cells, a BrdU incorporation assay was performed. Following treatment with siMed for 5 consecutive days following lentiviral infection, the results showed that the growth of SKOV-3 and HEY cells transduced with siMed was significantly inhibited compared with the siNon-transduced and parental cells (Fig. 3A and B). A 70% reduction in viability was noted in the SKOV-3 cells with MED19 gene silencing compared with the siNon group on day 5.
24 h, the rate of DNA synthesis was not significantly affected in the SKOV-3 and HEY cells. After 48 h of transduction with siMed, however, the DNA synthesis was suppressed by 39% in the SKOV-3 cells and 41% in the HEY cells compared to the cells that were transduced with siNon (Fig. 3C and D).

Knockdown of MED19 induces cell cycle arrest in ovarian cancer cells. Cellular DNA contents of parental, siNon-transduced and siMed-transduced cells were analyzed by fluorescence-activated cell sorting to determine their cell cycle status. The results showed that the siMed-transduced SKOV-3 cells formed fewer cells in each colony and 33% fewer colonies compared to the cells that were transduced with siNon (Fig. 3C and D).

As the HEY cells were not capable of forming colonies in this study, SKOV-3 cells were used to examine the effects of MED19 gene silencing on colony forming potential. The results showed that the siMed-transduced SKOV-3 cells formed fewer cells in each colony and 33% fewer colonies compared to the cells that were transduced with siNon (Fig. 3C and D).

Knockdown of MED19 inhibits the growth of ovarian cancer in vivo. The effect of MED19 gene silencing on the growth of ovarian cancer was analyzed in nude mice that were injected with siMed-transduced cells. Compared with the mice that were injected with parental and siNon-transduced cells, tumor growth was significantly delayed in the siMed group, which was evident from 2 weeks after transplantation until the day when the mice were sacrificed (Fig. 5). At day 42, the xenograft tumor weights were 372.5±277.6 mg in the mice injected with parental cells, 260.0±126.6 mg in the siNon group and 12.5±2.5 mg in the siMed group. No marked change in behavior, appearance or body weight was observed in the studied mice.

Discussion

Ovarian cancer is one of the most lethal gynecological malignancies. Although new chemotherapeutic agents have improved the survival rate for ovarian cancer patients over the past few decades, overall mortality from the disease still remains high. For patients in stage III and IV, the 5-year survival is approximately 34 and 18%, respectively (13-16). The reduced 5-year survival rates necessitate the need to develop an improved understanding of cell growth and death in ovarian cancer to identify new etiologic, prognostic or therapeutic targets.

MED19 is a component of the mediator complex, which is a co-activator for DNA binding factors that activate transcription via RNA polymerase II. Mediator is recruited to promoters by direct interactions with regulatory proteins and serves as a scaffold for the assembly of a functional pre-initiation complex.
Intraperitoneal nude mice were injected with parental, siNon- and siMed-infected cells. SKOV-3 and HEY, showed that the lentivirus-mediated diseases, including cancers. Thus, the combination of lentivirus and may be a potential therapeutic strategy for various RNAi is a useful tool for the functional analysis of specific and effective downregulation of target gene expression particularly since antisense technology allows for the highly therapeutic effects of gene silencing in a variety of diseases, gene delivery. Several studies have focused on evaluating the into the host cell and is one of the most efficient methods of is capable of delivering a large amount of genetic information characterized as having a long incubation period. Lentivirus, a member of slow viruses of the strong dependence on the efficiency of gene transfer. Lentivirus, The therapeutic efficiency of cancer gene therapy strategies innovative approach to the treatment of human cancer (17-20). MED19 expression in ovarian cancer. Whether other members of the mediator complex are abnormally regulated in human ovarian cancer or other human cancers remains unknown. The effectiveness of MED19 RNAi therapy in human ovarian cancer and other human cancer warrants further investigation.

Acknowledgements

This research was sponsored by the Fundamental Research Funds for the Central Universities of Fudan University.

References


