Lung Cancer

Role of Metallothionein1H in Cisplatin Resistance of Non-Small Cell Lung Cancer Cells

Xin-fang Hou[1*], Qing-xia Fan[2], Liu-xing Wang[2], Shi-xin Lu[3**]

[1]Department of Medicine, Henan Tumor Hospital, Zhengzhou 450002, China; [2]Cancer Center of Zhengzhou University; Department of Oncology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou 450052, China; [3]Department of Etiology and Carcinogenesis, Cancer Institute(Hospital), Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100021, China

CLC number: R734.2 Document code: A Article ID: 1000-9604(2009)04-0247-08
DOI: 10.1007/s11670-009-0247-9

ABSTRACT

Objective: Despite platinum-based adjuvant chemotherapy has improved greatly patients' outcomes, drug resistance poses a major impediment to the successful use of such an effective agent. Metallothioneins(MTs) are known to play putative roles in cancer cell proliferation, apoptosis, differentiation, drug resistance and prognosis. The present studiy was to investigte the role of metallethioein1H(MT1H) in cisplatin resistance of human non-small cell lung cancer(NSCLC) cell lines *in vitro* or its possible molecular mechanisms.

Methods: MT1H mRNA expression in A549 and A549/DDP cells was detected by RT-PCR. A recombinant eukaryotic expression plasmid pcDNA3.1(-)-MT1H was constructed and transfected into A549 cells which express no MT1H. MT1H siRNA was transfected into A549/DDP cells which express MT1H highly. MT1H expression was detected by RT-PCR and Immunoblot. The chemosensitivity to cisplatin was assessed by MTT assay. Apoptosis rate was determined by Tunel and FCM. Bcl-2 and Bax were determined by immunohistochemistry.

Results: MT1H mRNA was expressed in A549/DDP but not in A549. After transfection of MT1H, MT1H expression was enhanced and the chemosensitivity to cisplatin was decreased in A549 cells. Inversely, after transfection of MT1H siRNA, MT1H expression was decreased and the chemosensitivity to cisplatin was increased in A549/DDP. The apoptosis rate induced by cisplatin was increased and Bcl-2 was down-regulated but Bax showed little change in A549/DDP cells interferred with MT1H siRNA.

Conclusion: MT1H overexpression can promote drug resistance in A549 cells . Down-regulation of MT1H interfered with siRNA can effectively reverses the drug resistance in A549/DDP cells by down-regulating the expression of Bcl-2 and increasing cisplatin induced apoptosis. SiRNA targeting MT1H combined with chemotherapy may be a very promising strategy for treatment of lung cancer.

Keywords: Metallothionein; MT1H; Non-small-cell lung cancer; Drug resistance; Apoptosis

INTRODUCTION

Non-small-cell lung cancer accounts for 70%-80% of lung cancer. Chemotherapy is one of the main treatments. Despite platinum-based adjuvant chemotherapy has improved greatly patients' outcomes, drug resistance poses a major impediment to the successful use of such an effective agent. Therefore, to find a biomarker related with chemotherapy resistance and a potential target for cancer therapy is among the most important topics. With the development of research on gene therapy, gene therapy in combination with chemotherapy is becoming one of comprehensive treatments.

Received: Feb. 12, 2009; **Accepted:** July 22, 2009
This work was supported by the grants from State Key Basic Research "973" Programs of China (2002CB513101 and 2004BC518701)
*E-mail: hxinf@yahoo.com.cn
**Corresponding author.
E-mail: shlu@public.bta.net.cn

Metallothioneins(MTs) are a group of ubiquitous low molecular mass, cysteine-rich intracellular metal-binding proteins[1]. Recent years, the relationship between metallothionein(MT) and tumors has become a focus of research[2,3]. MTs are known to play putative roles in cancer cell proliferation, apoptosis, differentiation, drug resistance and prognosis[4-8]. In human, MT genes are located on chromosome 16q13 and may consist of at least 10 identified functional genes: MT2A[9], MT1A[10], MT1B[11], MT1E and MT1F[12], MT1G[13], MT1H and MT1X[14], MT3[15], MT4[16]. Researches revealed different MT genes in human possibly play different functional roles during development or under various physiological conditions[17]. MT isoforms may have unique functions[18].

The specific functional roles of each of the MT isoforms are not precisely known. So far, there are some data about MT2A isoform but lack of data on the function of MT1H. In the present study, whether MT1H is involved in cisplatin resistance NSCLC was explored. Cell models of under- or over-express MT1H were constructed to examine the effect of MT1H to cisplatin resistance in A549 and cisplatin-resistance A549 cells (A549/DDP). Further the possible mechanism underlying this drug resistance was investigated.

MATERIALS AND METHODS

Cell Culture

The MDR human non-small cell lung cancer cell line, A549/DDP, was supplied by Xiangya medical college. Its parental, sensitive line A549 was conserved in our institute. Cells were cultured in RPMI 1640 medium with 10% fetal bovine serum, 100 units/ml penicillin and 100 U/ml streptomycin at 37°C in a 5% CO_2 humidified atmosphere.

Plasmid Construction

MT1H cDNA fragment was PCR amplified with primers 5'-TGATCTAGAATGGACCCCAACTGCT CC-3'(sense) and 5'-GATGGATCCGGCACAGCAG CTGCAC-3'(antisense) using plasmid pACT2-MT1H as template. The PCR products were digested with XbaI and BamHI (Takara, Japan), and was cloned into the corresponding sites of pcDNA3.1(-)/myc-his (Invitrogen, USA). The construct was confirmed by restrictive digestion and DNA sequencing.

Cell Transfection

One day before transfection, cells were plated in 6-well plates and cultured up to 60%–80% confluence before transfection. According to the manufacturer's instruction, A549 cells were transfected with the eukaryotic expression vector alone pcDNA3.1(-)or with pcDNA3.1(-)-MT1H and A549/DDP cells were transfected with MT1H siRNA (Santa Cruz, USA) or control siRNA(Santa Cruz, USA) using Lipofectamine-2000 reagent(Invitrogen). After transfection for 48 h, MT1H level was detected by RT-PCR and Immunoblot and the sensitivities to DDP were verified by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Total RNA Isolation and RT–PCR

After transfection for 48 h, MT1H levels were measured by RT-PCR(Takara) in both cells. Total RNA was isolated from cells with Trizol (Invitrogen, USA) following the manufacturer's instructions. The quantity and purity of the RNA prepared from each sample were determined by UV absorbance spectroscopy. β-actin was used as an internal control. MT1H primers were 5' CCA GTC TCA CCT CGG CTT G 3'(sense) and 5'TAG CAA ATG AGT CGG AGT TGT 3'(antisense). β-actin primers were 5'CAT CCT GCG TCT GGA CCT 3'(sense) and 5'TCA GGA GGA GCA ATG ATC TTG 3'(antisense). PCR amplification was performed with 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 54°C, and 1 min extension at 72°C. PCR products were electrophoresed on 1.5% agarose gel and PCR fragments were visualized by ethidium bromide staining.

Western Blot

Cells were extracted in RIPA lysis buffer for subsequent immunoblotting studies. Protein concentration was determined using the Bradford assay[41]. Fifty mg protein was resolved by Tricine-sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then transferred to 0.2 μm PVDF membrane (Millipore, USE). Blocking was performed in Tris-buffered saline containing 5% dried fatfree milk and followed by overnight incubation with primary anti-6×His antibody(Zhongshan, China). As secondary antibodies, peroxidase-conjugated goat anti-mouse IgG were added for 1 h at room temperature. Visualization was performed using the DAB kit according to the manufacturer's instructions. The membranes were reprobed with 1: 300 polyclonal goat anti-actin, using the same procedure as described above.