Biomedicine & Pharmacotherapy 71 (2015) 172–184

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Increased sensitivity of human lung adenocarcinoma cells to cisplatin associated with downregulated contactin-1

Ruijie Zhang [a,1], Wei Yao [a,1], Pin Qian [a], Yingjie Li [b], Chaowen Jiang [a], Zhi Ao [a], Guisheng Qian [a], Changzheng Wang [a], Guoming Wu [a], Jin Li [a], Fuyun Ji [a,*], Jianping Xu [c,*]

[a] Institute of Human Respiratory Disease, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China
[b] Department of Endocrinology, the First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China
[c] Department of Pathology, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China

ARTICLE INFO

Article history:
Received 14 October 2014
Accepted 5 November 2014

Keywords:
CNTN-1
MDR
Cisplatin
Apoptosis
Invasion
Metastasis
Lung adenocarcinoma

ABSTRACT

Contactin-1 (CNTN-1), a glycosyl phosphatidylinositol anchor neural cell adhesion molecule (ACAM), is thought to function not only in nervous system development but also in the invasion and metastasis of several tumours. To investigate whether CNTN-1 is involved in multidrug resistance (MDR) in lung adenocarcinoma, CNTN-1 expression was compared between MDR human lung adenocarcinoma A549/cisplatin (A549/DDP) cells and its progenitor A549 cells. The comparison showed that CNTN-1 expression in A549/DDP cells was significantly higher than in A549 cells both at the mRNA level and the protein level. In order to confirm the physiological function of the abnormal expression, lentivirus-mediated short hairpin RNA (shRNA) was used to silence CNTN-1. Cell cytotoxicity assay and cell apoptosis assay revealed that silencing CNTN-1 both in A549 cells and in A549/DDP cells not only rendered cells more sensitive to cisplatin than the negative control, but also increased the cisplatin-induced apoptosis. Metastasis and invasion assays demonstrated that CNTN-1 knockdown reduced metastasis and invasion but did not affect A549 or A549/DDP cell proliferation. To investigate whether the abnormal expression of CNTN-1 is associated with characteristics of patients with non-small cell lung cancer (NSCLC), immunohistochemistry was used to detect CNTN-1 expression in 143 tissue samples from NSCLC patients and the results showed that the degree of CNTN-1 expression positively correlated with lymphatic invasion in patients with lung adenocarcinoma who received adjuvant cisplatin- or carboplatin-based treatment after surgery. Thus, we concluded that CNTN-1 is closely related with MDR of lung adenocarcinoma. Additionally, CNTN-1 is a novel marker to predict chemotherapeutic efficacy of patients with lung adenocarcinoma, especially with regard to cisplatin- or carboplatin-based regimens.

1. Introduction

Lung cancer is the leading cause of cancer mortality worldwide [1,2]. Non-small cell lung cancer (NSCLC) represents 85% of these tumours, and lung adenocarcinoma has become the major pathologic type of NSCLC [3,4]. Currently, increasing pollution and delayed diagnosis (most patients are identified at advanced stages of the disease and no longer have the option of surgical treatment) present challenges in reducing lung cancer mortality. Although targeted therapy has allowed for substantial progress in NSCLC treatment over the past decade, only a limited population [for example, patients with an epidermal growth factor receptor (EGFR) mutation in lung adenocarcinoma among female never-smokers in East Asia] benefits from these treatments [4–6]. Chemotherapy still plays an indispensable role in the standardised treatment of NSCLC, and cisplatin (DDP)-based chemotherapy is the standard first-line treatment for advanced, EGFR-wild-type lung adenocarcinoma. However, regretfully, acquired multidrug

Abbreviations: DDP, cisplatin; CNTN-1, Contactin-1; EGFR, epidermal growth factor receptor; IHC, Immunohistochemistry; MDR, multidrug resistance; NCAM, neural cell adhesion molecule; NMJ, neuromuscular junction; NSCLC, non-small cell lung cancer; OSCC, oral squamous cell carcinoma; P-gp/MDR1, P-glycoprotein; RT-PCR, reverse transcription PCR; shRNA, short hairpin RNA; VEGF-C, vascular endothelial growth factor C.

* Corresponding authors. Tel.: +862368755557; fax: +862368755544.
E-mail addresses: jifuyun@263.net, 923953263@qq.com (F. Ji), xujianping33@sina.com (J. Xu).

[1] Ruijie Zhang and Wei Yao contributed equally to this work.

http://dx.doi.org/10.1016/j.biopha.2014.11.004
0753-3322/

resistance (MDR) induced by chemotherapy remains a major barrier to successful chemotherapeutic treatment [7]. MDR in NSCLC is complex and multifactorial, involving heterogeneity [8], DNA repair [9,10], increased drug efflux [11], apoptosis [12], and the activation of detoxification systems [13]. These mechanisms of chemotherapy resistance generally result in poor clinical results for agents such as MDR blockers [14,15]. Thus, an understanding of other potential MDR mechanisms is essential to discover novel chemotherapy drugs and improve chemotherapy efficacy.

Contactin-1 (CNTN-1) is a member of the contactin subgroup of the immunoglobulin superfamily and maps to human chromosome 12q11-q12 [16]. Previous studies have revealed that CNTN-1 is a glycosyl phosphatidylinositol anchor neural cell adhesion molecule (NCAM) that is differentially expressed in numerous neuronal tissues and thought to function in nervous system development [17]; the loss of CNTN-1 from the neuromuscular junction (NMJ) impairs communication or adhesion between nerve and muscle and was proposed to result in severe myopathies [18]. Subsequent study revealed that CNTN-1 was associated with invasive ability and to inversely correlate with the prognosis of lung adenocarcinoma patients, and it was proposed to function in the invasion and metastasis of lung adenocarcinoma cells via RhoA-mediated mechanisms [19]. Studies in oesophageal squamous cell carcinoma [20,21], oral squamous cell carcinoma [22], and gastric cancer [23] further confirmed that CNTN-1 plays a critical role in the development, progression, and pathogenesis of these tumours.

MDR, invasion, and metastasis are major barriers to the successful treatment of lung cancer. To investigate whether CNTN-1 is involved in MDR in lung adenocarcinoma, its expression was compared between MDR human lung adenocarcinoma A549/cisplatin (A549/DDP) cells and the progenitor A549 cells in the study. This comparison revealed that CNTN-1 expression in A549/DDP cells was significantly higher than in A549 cells. Silencing CNTN-1 in A549 and A549/DDP cells not only rendered both cell lines more sensitive to cisplatin, but also increased the cisplatin-induced apoptosis compared with the negative control shRNA-treated cells. Furthermore, the degree of CNTN-1 expression was found to be positively correlated with lymphatic invasion in patients with lung adenocarcinoma who received adjuvant cisplatin- or carboplatin-based treatment after surgery.

2. Patients, materials and methods

2.1. Cell lines

Lung adenocarcinoma cell line A549 were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China) and cultured in RPMI 1640 medium (Gibco, Lot# 8114040). The medium was supplemented with 10% foetal bovine serum (FBS; Gibco, Lot# 1227694) and 1% penicillin-streptomycin (Hyclone, Lot# SV30010). The MDR cell line A549/DDP was established as described by Guo et al. [24]. Briefly, the progenitor A549 cells were treated with a high-dose shock of cisplatin (1.0 μg/mL) and then selected for more than 6 months with increasing concentrations of cisplatin from 0.05 to 1.0 μg/mL in a 37 °C humidified incubator with 5% CO_2. The selected cells, A549/DDP, which demonstrated cross-resistance to hydroxycamptothecin, vincristine, and 5-fluorouracil, and the progenitor cells were regularly maintained in medium with FBS and penicillin-streptomycin in a 37 °C humidified incubator with 5% CO_2. Upon reaching 90% confluence, the cells were passaged using 0.25% trypsin with 0.1% EDTA (Gibco, Lot# 1391607).

2.2. Patients and specimens

Tissue samples were collected between 2010 and 2011 from 143 primary lung carcinoma patients who had not received chemotherapy or radiotherapy prior to surgery (mainly adenocarcinoma, squamous cell carcinoma, and adenosquamous carcinoma) at Xinqiao Hospital, Third Military Medical University. Five-micrometer tissue sections of paraffin-embedded, formalin-fixed surgical specimens were collected from the Department of Pathology of Xinqiao Hospital for immunohistochemical staining. The study was approved by the Ethics Committee of Xinqiao Hospital, Third Military Medical University. After receiving an explanation of the study purpose and procedures, all participants signed an informed consent form and completed a detailed questionnaire regarding their gender, age, and smoking habits. All diagnoses were based on pathological and/or cytological evidence. The histological classification of lung cancer was determined as recommended by WHO [25].

2.3. Gene expression by reverse transcription PCR (RT-PCR)

Total RNA was isolated using TRIzol according to the manufacturer's recommendations (Invitrogen, Lot# 15596-026). A total of 1 μg was reverse transcribed using the PrimeScript® RT reagent Kit with gDNA Eraser (Perfect Real Time) according to the manufacturer's instructions (TaKaRa Biotechnology Co. Ltd., Lot# AK2501). Gene expression levels were normalised to the internal control β-actin. The CNTN-1 forward primer was 5′- GCCCATGACAAAGAAGAAGC-3′, and the reverse primer was 5′-CGACATGATCCCAGGTGATT-3′. The β-actin forward primer was 5′-CCTGGCACCCAGCACAAT-3′, and the reverse primer was 5′-GGGCCGGACTCGTCATAC-3′. The conventional RT-PCR was performed as previously described [26]. Briefly, three-step PCR was performed according to the system manual of the PCR apparatus (GeneAmp PCR system 9700, Applied Biosystems). The total reaction volume was 20 μL, including 2 μL of 10× PCR buffer, 0.6 μL of 10 mM dNTPs, 0.6 μL of 10 μM forward primers, 0.6 μL of 10 μM reverse primers, 2 μL of cDNA, 0.5 μL of 2.5 U/mL Taq DNA Polymerase, and 13.7 μL of RNase-free H_2O. The PCR cycling conditions included a single pre-denaturation step at 94 °C for 4 min; 35 cycles of denaturation at 94 °C for 40 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 1 min; and a final incubation at 72 °C for 10 min. PCR products were separated by electrophoresis on a 2.5% agarose gel and visualised by staining with 0.5% ethidium bromide.

2.4. Western blot analysis

Cell lysates were prepared using M-PER® Mammalian Protein Extraction Reagent (Thermo Scientific, Pierce Biotechnology, Lot# OF185817) with complete ULTRA Tablets (Mini, EDTA-free, EASY pack, Roche Diagnostics GmbH, Lot# 04693159001). A total of 100 μg of cell lysate was separated on a 10% SDS-PAGE gel (Beijing Dingguo Changsheng Biotech. Co., Ltd, Lot# 33300120) and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Lot# ISEQ00010). The membranes were blocked with 5% bovine serum albumin (BSA; Genview, Lot# 3318030150) and then incubated with the indicated antibodies at 4 °C overnight. Appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies were incubated for 1 h at room temperature. Signals were detected using a SuperSignal® West Pico Chemiluminescent Substrate (Thermo Scientific, Pierce Biotechnology, Lot# LG144469A). The primary and secondary antibodies and the concentrations used were as follows: anti-CNTN-1 (1 μg/mL, Abcam, Lot# ab101866); anti-β-actin mouse monoclonal antibodies (5B7; 1:1000, Beijing TDY Biotech CO., Ltd., Lot# TDY041F), goat polyclonal secondary antibody to mouse IgG-H&L (HRP;