European Review for Medical and Pharmacological Sciences 2020; 24: 8830-8836

LncRNA HMGA1P4 promotes cisplatin-resistance in gastric cancer

X.-L. QIAO[1], Z.-L. ZHONG[1], Y. DONG[1], F. GAO[2]

[1]Department of Gastroenterology, The Second Affiliated Hospital of Dalian Medical University, Dalian, China
[2]Comprehensive Intervention Division, The Second Affiliated Hospital of Dalian Medical University, Dalian, China

Xiuli Qiao and Zhiling Zhong contributed equally to this work

Abstract. – OBJECTIVE: Long non-coding RNA (lncRNA) HMGA1P4 has been previously reported to be upregulated in gastric cancer (GC). This study aims to investigate the role of HMGA1P4 in cisplatin (DDP)-resistant GC.

PATIENTS AND METHODS: HMGA1P4 levels in DDP-resistant GC tissues and cells were determined. Regulatory effects of HMGA1P4 on proliferative and apoptotic abilities in DDP-resistant GC cells and their parental cells were assessed. At last, expression levels of genes associated with multidrug-resistance (MDR) (MDR1, MRP1, mTOR and HIF-1α) and apoptosis (Bax, Bcl-2 and Caspase3) were determined in DDP-resistant GC cells.

RESULTS: Results revealed that HMGA1P4 was upregulated in DDP-resistant GC tissues and cells. Overexpression of HMGA1P4 stimulated proliferative rate and suppressed apoptosis in both DDP-resistant GC cells and their parental cells. Moreover, in DDP-resistant GC cells, overexpression of HMGA1P4 upregulated MDR-related genes and downregulated apoptosis-related genes.

CONCLUSIONS: HMGA1P4 is upregulated in DDP-resistant GC tissues and cells, and triggers the progression of DDP-resistance in GC.

Key Words:
HMGA1P4, Gastric cancer (GC), DDP-resistance, Proliferation, Apoptosis.

Introduction

Globally, the mortality of gastric cancer (GC) ranks second in all malignancies. Overall prognosis of GC is unsatisfactory, although great strides have been made in diagnostic and therapeutic methods. Generally speaking, the 5-year survival rate of GC is about 20-30%. Surgery combined with postoperative chemotherapy is preferred for preventing metastases and recurrence, as well as prolonging survival of GC[1]. Survival of advanced GC is extremely short, with the median survival of less than 10 months[2]. 5-fluorouracil (5-FU) accompanied with cisplatin (DDP) is recognized as the first-line chemotherapy regimen for advanced GC. Nevertheless, drug-resistance severely limits therapeutic efficacy in GC, leading to recurrence and metastases[3], so it is necessary to uncover the mechanisms of DDP-resistance in GC, thus improving therapeutic outcomes of GC patients.

Long non-coding RNAs (lncRNAs) are a type of endogenous RNAs without protein-encoding function[4]. They were initially considered as transcription noise. Recently, vital functions of lncRNAs in cellular behaviors have been identified[5]. Non-coding transcripts, including lncRNAs, lack the open reading frame and/or conservative codon. Recent studies have uncovered the potential of lncRNAs to translate and produce small peptide. The secondary structure of lncRNA decides its biological function through binding to proteins, reconstitute chromatin or regulating transcription factors. Meanwhile, lncRNAs are able to indirectly affect mRNA expressions through corresponding miRNAs, or directly influence transcription, shear and degradation of miRNAs[6]. In summary, lncRNAs exert diverse regulatory effects on life activities.

In the therapeutic strategies of malignant tumors, DDP-based chemotherapy is extensively applied, showing a fundamental place in tumor treatment. Several lncRNAs are reported to influence drug-resistance in tumor diseases. For

Corresponding Authors: Fei Gao, MM; e-mail: gaofei_19790813@163.com
Ying Dong, MM; e-mail: dongying0721@dmu.edu.cn

example, lncRNA HCP5 triggers DDP-resistance in adult triple negative breast cancer *via* regulating PTEN level[7]. PVT1/miR-216b/Beclin-1 regulatory loop affects DDP-sensitivity in NSCLC through mediating autophagy and apoptosis of tumor cells[8]. Downregulated lncRNA HOTTIP suppresses proliferation and enhances DDP-sensitivity in prostate cancer *via* activating the Wnt pathway[9]. Zhang et al[10] demonstrated that lncRNA HMGA1P4 is upregulated in GC. In this paper, the regulatory effect of HMGA1P4 on DDP-resistant GC and the underlying mechanism were mainly investigated.

Patients and Methods

Collection of Samples

Effective DDP treatment (DDP-sensitive GC) was defined as the reduction of primary tumors or malignant pleural effusion after DDP treatment in GC patients. If primary tumors were expanded, malignant pleural effusion increased or tumor recurred at 6-15 months following DDP treatment, DDP treatment in GC patients (DDP-resistant GC) was considered to be a failure. Tumor samples were collected from The Second Affiliated Hospital of Dalian Medical University and immediately preserved at -80°C. This investigation was approved by the Medical Ethics Committee of The Second Affiliated Hospital of Dalian Medical University. The written informed consent was obtained from patients before the study. This research was conducted in accordance with the Declaration of Helsinki. In all cases, the diagnoses and grading were confirmed by two experienced pathologists and were performed according to the criteria of the American Joint Committee on Cancer.

Cell Culture

MGC803 and SGC7901 cells purchased from American Type Culture Collection (ATCC; Manassas, VA, USA) were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640; HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 100 U/mL penicillin-streptomycin. DDP-resistant GC cells MGC803/DDP and SGC7901/DDP were induced by 12-month DDP treatment in their parental cells from the gradually increased dose of 0.05 mg/ml to 1 mg/mL. Prior to experiment, MGC803/DDP and SGC7901/DDP cells were incubated in DDP-free RPMI-1640 for 2 weeks.

Cell Transfection

HMGA1P4-overexpression lentiviral vector (LV-HMGA1P4) was obtained from GenePharma (Shanghai, China). Cells were transfected using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). At 6 hours, medium containing 10% FBS was replaced, and the cells after 48 h of transfection were harvested for other experiments.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA in cells and tissues was extracted using TRIzol method (Invitrogen, Carlsbad, CA, USA) for reverse transcription according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). RNA concentration was detected using a spectrometer. QRT-PCR was then performed based on the instructions of SYBR Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan). Relative level was calculated using $2^{-\Delta\Delta Ct}$ method. Primer sequences are as follows: HMGA1P4 Forward: 5'-ATCTGCGGGGGTGCTTATTC-3', Reverse: 5'-TTCCTGTACCCCAAAGGGGA-3', MDR1 Forward: 5'- ACCAAGCGGCTCCGATACA-3 ', Reverse: 5'-TCATTGGCGAGCCTGGTAGTC-3', MRP1 Forward: 5'-GGACCTGGACTTCGTTCTCA-3', Reverse: 5'- CGTCCAGACTTCATCCG-3', mTOR Forward: 5'-CCCGAGACAGCCTTGGCAGTTGG-3', Reverse: 5'-CAGGACTCAGGACACAACAGCCC-3', HIF-1α Forward: 5'-CTATGGAGGCCAGAAGAGGGTAT-3', Reverse: 5'-CCCACATCAGGTGGCTCATAA-3' and GAPDH Forward: 5'-CGGAGTCAACGGATTTGGTCGTAT-3', Reverse: 5'-AGCCTTCTCCATGGTGGTGAAGAC-3'.

5-Ethynyl-2'-Deoxyuridine (EdU) Assay

Cells were inoculated into 96-well plates with 1×10^5 cells per well and labeled with 100 μL of EdU reagent (50 μM) per well for 2 h. After washing with phosphate-buffered saline (PBS), the cells were fixed in 50 μL of fixation buffer, decolored with 2 mg/mL glycine and permeated with 100 μL of penetrant. After washing with PBS once, cells were stained with Hoechst33342 in the dark for 30 min. EdU-positive ratio was determined under a fluorescent microscope.

Flow Cytometry

Cells were washed with PBS twice, digested and suspended in binding buffer. Subsequently, cells were incubated with 5 μL of AnnexinV-FITC

(fluorescein isothiocyanate) and 5 μL of Propidium Iodide (PI) at room temperature in the dark. Apoptotic rate was examined by flow cytometry on EPICS XL-MCL FACScan (Becton-Dickinson, Mountain View, CA, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM, Armonk, NY, USA) was used for data analysis. GraphPad Prism 7 (La Jolla, CA, USA) was utilized for depicting figures. Data were expressed as mean ± standard deviation ($\bar{x}$±SD). Differences between the two groups were compared using the *t*-test. $p<0.05$ suggested that the difference was statistically significant.

Results

HMGA1P4 Is Involved In DDP-Resistance In GC

GC tissues were harvested from DDP-resistant and DDP-sensitive patients with GC. QRT-PCR data showed a higher abundance of HMGA1P4 in DDP-resistant GC tissues than those of sensitive ones (Figure 1A). In addition, HMGA1P4 level was similarly upregulated in DDP-resistant GC cell lines relative to their parental cell lines (Figure 1B). It is suggested that HMGA1P4 is involved in DDP-resistance in GC.

Overexpression of HMGA1P4 Triggers Drug Resistance in DDP-Resistant GC Cells

To uncover the influence of HMGA1P4 in drug-resistant GC, LV-HMGA1P4 was constructed. Transfection of LV-HMGA1P4 remarkably upregulated HMGA1P4 level in MGC803/DDP and SGC7901/DDP cells (Figure 2A). Moreover, DDP treatment in drug-resistant GC cells overexpressing HMGA1P4 presented higher an EdU-positive ratio and a lower apoptotic rate than those transfected with controls. Compared with controls, transfection with si-HMGA1P4 markedly inhibited proliferative potential and induced apoptosis in MGC803/DDP and SGC7901/DDP cells (Figure 2B-2E). It can be considered that HMGA1P4 triggers DDP-resistance in GC through promoting proliferation and attenuating apoptosis of tumor cells.

Overexpression of HMGA1P4 Promoted Proliferation and Attenuated Apoptosis In GC

Furthermore, proliferative and apoptotic changes in parental GC cells influenced by HMGA1P4 were assessed. The results showed that transfection with LV-HMGA1P4 increased EdU-positive ratio and decreased apoptotic rate in MGC803 and SGC7901 cells (Figure 3A). On the contrary, knockdown of HMGA1P4 attenuated proliferative capacity and stimulated apoptosis in MGC803 and SGC7901 cells (Figure 3B-3D).

HMGA1P4 Regulated Genes Associated With Multidrug-Resistance (MDR) and Apoptosis

Expression levels of MDR-associated genes (MDR1, MRP1, mTOR and HIF-1α) and apoptosis-associated genes (Bax, Bcl-2 and Caspase3) were determined in DDP-resistant GC cells. It was found that transfection with LV-HMGA1P4

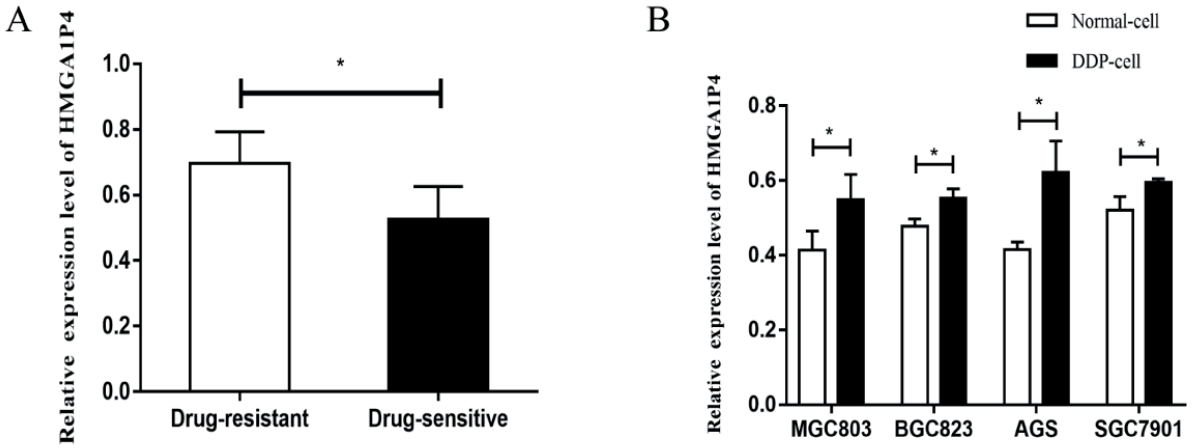

Figure 1. HMGA1P4 is involved in DDP-resistance in GC. **A,** HMGA1P4 level in DDP-resistant (n=15) or DDP-sensitive (n=15) GC tissues. **B,** HMGA1P4 level in DDP-resistant GC cells and their parental cells. Data are presented as mean ± SD, *$p<0.05$.

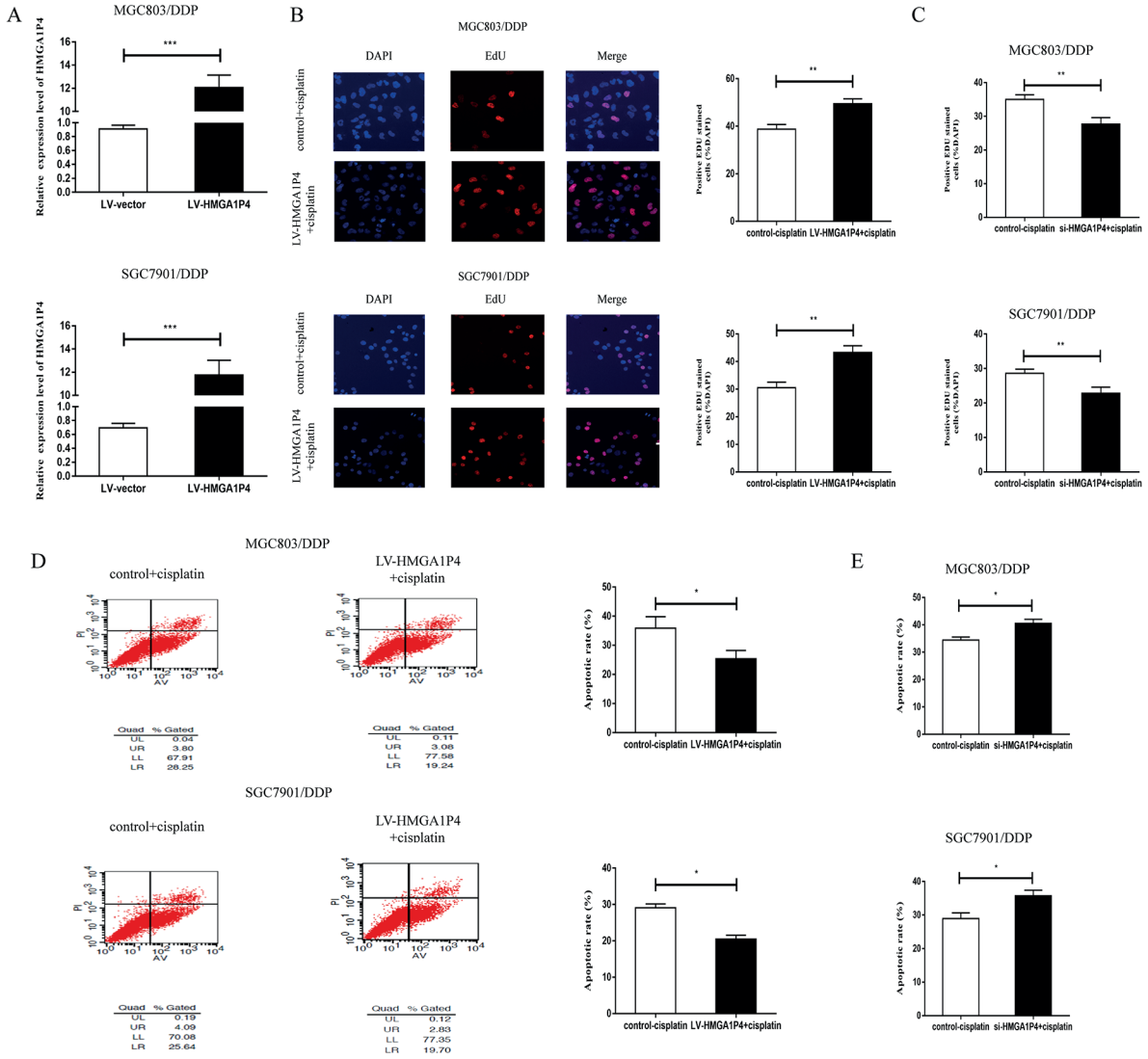

Figure 2. Overexpression of HMGA1P4 triggers drug resistance in DDP-resistant GC cells. **A,** Transfection efficacy of LV-HMGA1P4 in MGC803/DDP and SGC7901/DDP cells. **B,** EdU-positive ratio in DDP-treated MGC803/DDP and SGC7901/DDP cells transfected with LV-HMGA1P4 or control (magnification: 20×). **C,** EdU-positive ratio in DDP-treated MGC803/DDP and SGC7901/DDP cells transfected with si-HMGA1P4 or control. **D,** Apoptotic rate in DDP-treated MGC803/DDP and SGC7901/DDP cells transfected with LV-HMGA1P4 or control. **E,** Apoptotic rate in DDP-treated MGC803/DDP and SGC7901/DDP cells transfected with si-HMGA1P4 or control. Data are presented as mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

in DDP-treated MGC803/DDP and SGC7901/DDP cells markedly upregulated MDR1, MRP1, mTOR and HIF-1α (Figure 4A). Besides, Bcl-2 and Caspase3 were upregulated, while Bax was downregulated in DDP-treated drug-resistant GC cells overexpressing HMGA1P4 (Figure 4B). Besides, the protein level of these two cell lines was according to Figure 4C. It is further verified that HMGA1P4 stimulates DDP-resistance in GC *via* accelerating MDR and inhibiting apoptosis.

Discussion

GC is a prevalent malignancy in the digestive tract, whose incidence is secondary to lung cancer[11]. The occurrence and progression of GC are gradual carcinogenic processes involving multiple factors. Currently, low detective rate of early-stage GC, poor survival and high rates of recurrence and metastases severely restrict the therapeutic efficacy of GC[12]. Therefore, chemotherapy is critical for GC patients, especially

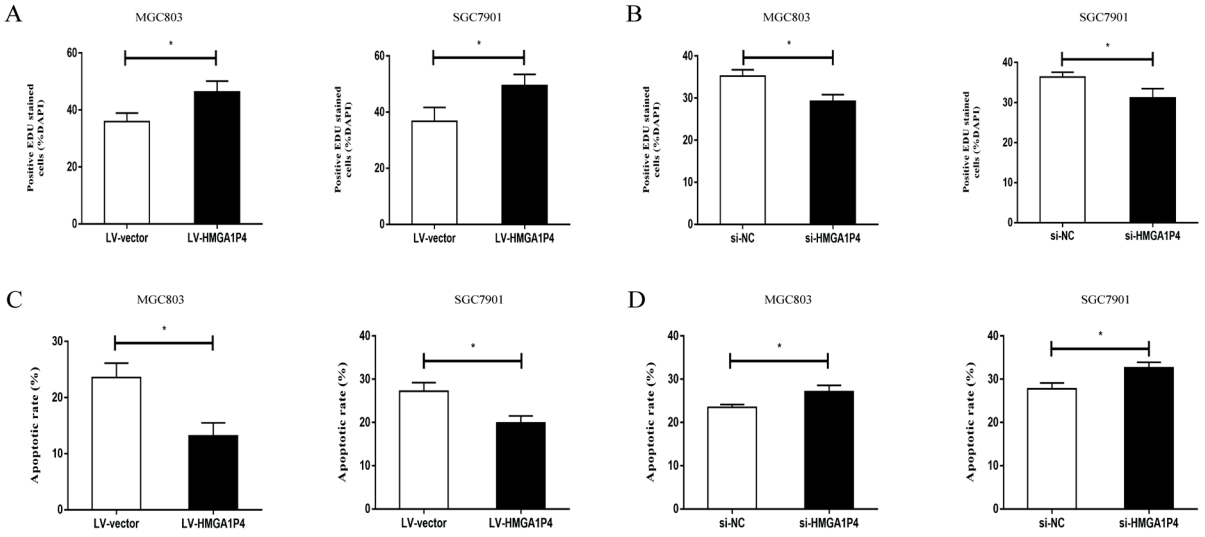

Figure 3. Overexpression of HMGA1P4 promotes proliferation and attenuates apoptosis in GC. **A,** EdU-positive ratio in DDP-treated MGC803 and SGC7901 cells transfected with LV-HMGA1P4 or control. **B,** EdU-positive ratio in DDP-treated MGC803 and SGC7901 cells transfected with si-HMGA1P4 or control. **C,** Apoptotic rate in DDP-treated MGC803 and SGC7901 cells transfected with LV-HMGA1P4 or control. **D,** Apoptotic rate in DDP-treated MGC803 and SGC7901 cells transfected with si-HMGA1P4 or control. Data are presented as mean ± SD, *$p<0.05$.

those in advanced stage. Nevertheless, chemotherapy-resistance (especially MDR) is an obstacle in the treatment[13].

Drug-resistance of tumor cells is one of the defense mechanisms for maintaining their stability and growth, and is also a key factor leading to tumor recurrence, metastasis and poor treatment outcomes[14,15]. The two basic characteristics of drug resistance: firstly, it changes and interferes with the absorption, distribution, efflux and metabolism of drugs. Secondly, it changes the cytotoxic abilities, including mutations of target molecules, cell cycle arrest, increased DNA repair ability and apoptosis inhibition[16,17]. In addition, the interaction of tumor cell matrix and tumor location may also contribute to the occurrence of MDR. Multiple factors and pathways are involved in drug-resistance of tumor cells, including P-gp, topoisomerase, apoptosis-related genes and cytokines[18,19]. Recently, lncRNAs are discovered to be closely linked to MDR. For example, LINC00460 triggers gefitinib-resistance in NSCLC through sponging miR-769-5p to upregulate the epidermal growth factor receptor[20]. LncRNA OIP5-AS1 induces DDP-resistance in osteosarcoma by absorbing miR-340-5p to activate the LPAATβ/PI3K / AKT / mTOR pathway[21]. In liver cancer, lncRNA KCNQ1OT1 regulates Oxaliplatin-resistance *via* the miR-7-5p / ABCC1 axis[22].

Cisplatin is a metal platinum complex and a cyclic non-specific anti-tumor drug[23]. It rapidly dissociates in the low-chlorine environment, and combines with intracellular DNA in the form of hydrated cations to form interchain, intrachain or protein DNA crosslinks, thereby destroying the structure and function of DNA. Cisplatin is characterized by broad anti-cancer spectrum, strong anti-tumor effect and synergism with several anti-tumor drugs, which has been widely applied in chemotherapy[24,25]. However, the occurrence of DDP-resistance markedly influences therapeutic outcomes. DDP-resistance in GC is complex, involving multiple genes and factors[26].

Our findings uncovered that HMGA1P4 was upregulated in DDP-resistant GC tissues and cell lines. In addition, overexpression of HMGA1P4 was able to accelerate proliferative ability and inhibit apoptosis in DDP-resistant GC cells. Previous studies have demonstrated that most chemotherapeutic drugs exert their anti-tumor activity by inducing apoptosis or programmed cell death. Resistance to apoptosis may be a major factor leading to tumor treatment failure. Subsequently, regulatory effects of HMGA1P4 were identified on mediating expression levels of MDR-associated and apoptosis-associated genes. To sum up, HMGA1P4 influences DDP-resistance in GC mainly by targeting proliferative and apoptotic abilities.

Conclusions

HMGA1P4 is upregulated in DDP-resistant GC tissues and cells. It triggers the progression of DDP-resistance in GC, and may be utilized as a hallmark for monitoring the progression of drug-resistance.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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