LncRNA MALAT1 Regulates the Progression and Cisplatin Resistance of Ovarian Cancer Cells via Modulating miR-1271-5p/E2F5 Axis

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Background: Long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) were reported to be related to the development of ovarian cancer (OC). In this study, the functional mechanisms of lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) and microRNA-1271-5p (miR-1271-5p) were explored in OC.

Methods: The level of MALAT1, miR-1271-5p, or E2F transcription factor 5 (E2F5) was detected by qRT-PCR. MTT assay, flow cytometry analysis and transwell migration and invasion assays were performed to determine cell proliferation, apoptosis, migration and invasion, respectively. E2F5 protein expression was detected by Western blot. The interaction between miR-1271-5p and MALAT1 or E2F transcription factor 5 (E2F5) was confirmed by the dual-luciferase reporter assay.

Results: MALAT1 and E2F5 level were increased, while miR-1271-5p level was decreased in cisplatin (DDP)-resistant OC tissues and cells. MALAT1 knockdown or miR-1271-5p upregulation decreased IC_{50} of cisplatin, and inhibited cell proliferation, migration, invasion, and facilitated cell apoptosis in DDP-resistant OC cells. Moreover, MALAT1 sponged miR-1271-5p to upregulate E2F5 expression. Besides, MALAT1 knockdown decreased DDP resistance, inhibited cell proliferation, migration, invasion, and promoted cell apoptosis by sponging miR-1271-5p to downregulate E2F5 expression in DDP-resistant OC cell.

Conclusion: We demonstrated that MALAT1 mediated DDP-resistant OC development through miR-1271-5p/E2F5 axis, providing the theoretical basis for OC therapy.

Keywords: ovarian cancer, MALAT1, miR-1271-5p, E2F5, cisplatin

Introduction

Ovarian cancer (OC) is one of the most lethal tumors in female with approximately 140,000 deaths per year.\(^1\) Over 70% of OC patients were diagnosed at advanced stage, and the 5-year-survival rate was low in OC patients.\(^2,3\) Nowadays, the combination of surgery and chemotherapy is an effective method for OC therapy. Cisplatin (DDP) is considered as a good chemotherapeutic agent to repress the development of OC.\(^4\) However, it is easy to generate DDP resistance for OC patients. Thus, it is of great significance to explore the regulatory mechanism of drug resistance in OC.

Long noncoding RNAs (lncRNAs), with more than 200 nucleotides in length, are defined as a conserved family in RNAs that modulate the levels of downstream genes through various methods, such as epigenetic modification, transcriptional regulation, or post-transcriptional regulation.\(^5\) Amounting evidence demonstrated...
that IncRNAs were related to drug resistance in multiple human cancers, including glioma, breast cancer, colorectal cancer, and ovarian cancer. For instance, IncRNA metastasis associated with lung adenocarcinoma transcript 1 (MALAT1) depletion enhanced chemo-sensitivity of OC cells against DDP by regulating the Notch1 pathway. This result suggested that MALAT1 exerted an important role in DDP-induced OC development. However, the functional mechanism of MALAT1 in DDP-regulated OC progression is not fully clear.

MicroRNAs (miRNAs) are a group of small noncoding RNAs with about 20 nucleotides that affect the level of target genes through suppressing transcription or inducing the degradation of mRNAs. Previous studies indicated that the levels of miRNAs were abnormally expressed in various human cancers, revealing that miRNAs can be considered as a type of biomarkers for the diagnosis of cancers. MicroRNA-1241-5p (MiR-1271-5p), an endogenous miRNA, was lowly expressed in OC tissues according to the reporter in 2014. Moreover, miR-1271 repressed the growth of OC cells and weakened cell resistance to DDP. More importantly, a previous study indicated that MALAT1 could target miR-1271-5p to participate in the tumorigenesis of multiple myeloma. However, the role and underlying mechanism of miR-1271-5p in DDP resistance of OC cells is largely unknown.

E2F transcription factor 5 (E2F5) belongs to E2F family that plays an important role through modulating the levels of downstream genes in various cell progression, such as cell proliferation, mobility, and apoptosis. Increasing studies demonstrated that the main function of E2F5 was related to the promotion of cell cycle. E2F5 was upregulated in OC, and E2F5 promoted cell proliferation, mobility, and induced apoptosis. Moreover, the data showed that miR-1271-5p suppressed E2F5 to inhibit OC progression. These data indicated that E2F5 was a positive regulator for the development of OC. However, whether E2F5 was involved in DDP-resistance of OC progression is unclear.

In this study, we measured MALAT1, miR-1271-5p, and E2F5 expression in DDP-resistant OC tissues and cells. Furthermore, the functions of MALAT1 and miR-1271-5p in DDP-resistant cells were explored. Moreover, we investigated the association between miR-1271-5p and MALAT1 or E2F5. Besides, we explored whether MALAT1 regulated miR-1271-5p and E2F5 to modulate DDP-resistant OC progression.

### Materials and Methods

#### Samples Collection and Cell Culture

Twenty-five tumor-responsive OC tissues and 34 tumor-resistant OC tissues were obtained from patients with or without DDP resistance at the hospital of The First People’s Hospital of Lianyungang. The clinicopathologic features of OC patients with DDP resistance are presented in Table 1. The research was approved by the Ethics Committee of The First People’s Hospital of Lianyungang. All patients in this research signed informed contents and consent was obtained from the study participants prior to study commencement (approval number: 2019年审 (33) 号).

SKOV3 and OVCAR3 cell lines were bought from Biomedical Science cell bank (Shanghai, China). Complete Dulbecco’s Modified Eagle Medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, MA, USA) was used to culture cells at 37°C with 5% CO2. To generate DDP-resistant OC cells, SKOV3 and OVCAR3 cells were induced by DDP purchased from Sigma-Aldrich (St Louis, MO, USA). To generate SKOV3/DDP and OVCAR3/DDP cells, TSKOV3 and OVCAR3 cells were treated with DDP (0.1, 0.2, 0.5, 1 and 2 μg/mL) and the treatment was repeated 5 times for each concentration. After culture for 48 h, the medium was discarded and the fresh medium was added. After 12 months, the stable OC cell lines SKOV3/DDP and OVCAR3/DDP resistant to DDP were constructed at 2 μg/mL, and the cell lines were cryopreserved in liquid nitrogen. This research was carried out in accordance with the World Medical Association Declaration of Helsinki.

#### Table 1 The Clinicopathologic Features of OC Patients with DDP Resistance

| Parameters               | Groups | Numbers (n = 34) |
|--------------------------|--------|-----------------|
| Age                      | ≤50    | 12              |
|                          | >50    | 22              |
| TNM stage                | I+II   | 16              |
|                          | III+IV | 18              |
| Tumor size               | ≤4cm   | 15              |
|                          | >4cm   | 19              |
| Lymph node metastasis    | Negative | 20          |
|                          | Positive | 14           |

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Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from tissues and cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was acquired through Prime Script TM RT reagent kit (Takara, Dalian, China) complying with the constructor’s instruction. The PCR reaction was performed using SYBR® Premix Ex Taq™ II Kit (Takara) and conducted using ABI Step One Real-time PCR System (Thermo Fisher Scientific). Data were analyzed by the 2−ΔΔCt approach and normalized by Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6. The primers (Sangon, Shanghai, China) were listed as follows: TCACTGCCACCCAGAAGAC (antisense) and TCTCAGCACTTGGCA (sense) for MALAT1, CAGCAGTTGGCA CCTAGCA (sense) and TATGGTTGTCTCTCTCTG TCTC (antisense) for miR-1271-5p, CGGCGTCTGGATGC TCAA (sense) and CAATCCCCTCTAGACATTGTG (antisense) for E2F5, TGCCGGTGCTGCTCTCGGCCAG (sense) and CCAGTGCAAGGTCCGAGGT (antisense) for U6, ATACACTGCCACCCAGAAGAC (sense) and TTTCTA GACGGAGGTCAG (antisense) for GAPDH.

Cell Transfection

siRNA targeting MALAT1 (si-MALAT1#1 and si-MALAT1 #2), siRNA negative control (si-NC), pcDNA, and E2F5 were bought from GenePharma (Shanghai, China). miR-1271-5p mimics (miR-1271-5p) and miR-NC, and miR-1271-5p inhibitor (anti-miR-1271-5p) and anti-miR-NC were purchased from RIBOBIO (Guangzhou, China). Cell transfection was performed using Lipofectamine 3000 (Invitrogen).

MTT Assay

SKOV3/DDP and OVCAR3/DDP cell viability was determined by MTT Kit (Beyotime, Shanghai, China). The cells were added with 10 μL MTT for 4 h and incubated with dimethyl sulfoxide (DMSO) (Sigma-Aldrich) for 2 h. The optical density (OD) value was determined at 490 nm using a spectrophotometer.

Cell Apoptosis Assay

Cell apoptotic rate was detected via flow cytometry. 2 × 10⁵ cells transfected SKOV3/DDP and OVCAR3/DDP cells were placed into 6-well plates. Then, cells were harvested and incubated in binding buffer. Cells were incubated with Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) (Solarbio) for 15 min. Finally, cell apoptosis was measured using a flow cytometer (Agilent, Hangzhou, China). The apoptotic rate was presented as the percentage of cells (Annexin V-FITC+ and PI+).

Transwell Assesment

To detect the migrated ability, cells (1 × 10⁶ cells/well) in medium without serum were seeded into the upper chambers (Costar, Corning, NY, USA). The medium containing 10% serum was added in lower chamber. After 24 h, cells were fixed, stained with 0.5% crystal violet (Beyotime), and counted. Three random fields were selected. For invasion assay, cells (5 × 10⁴ cells/well) were placed into the upper chambers pre-coated with Matrigel (BD Bioscience, San Jose, CA, USA), and the other procedures were same as those in migration assay.

Dual-Luciferase Reporter Assay

The wild type and mutant type of MALAT1 or E2F5 3‘UTR (MALAT1-WT/MUT or E2F5 3‘UTR-WT/MUT) were cloned into pGL3 vector (Promega), and then co-transfected to SKOV3/DDP and OVCAR3/DDP cells with miR-1271-5p or miR-NC. The luciferase activity was determined by the dual-luciferase assay system (Promega) in line with the recommended protocol.

Western Blot Assay

The protein was quantified via NanoDrop Spectrophotometer and adjusted the concentration to 5 μg/μL. 10 μL of protein samples were loaded on each lane and subjected to SD-PAGE. Then, the protein was transferred onto the nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). Membranes were incubated with the primary antibodies overnight at 4°C and secondary antibody for 2 h. The antibodies included anti-E2F5 (1:1000, ab22855, Abcam, Cambridge, MA, USA), anti-GAPDH (1:2500, ab181602, Abcam) and horseradish peroxidase-conjugated IgG (ab205718, 1:20,000 dilution). The signals were observed using enhanced chemiluminescence (Thermo Fisher) and exposed to X-ray films. The gray values of bands were measured and the relative protein abundance was normalized to GAPDH expression.

Statistical Analysis

Statistical analysis was conducted via GraphPad Prism 6 software (GraphPad, La Jolla, CA, USA). Data were presented as mean ± standard deviation and analyzed by SPSS 22.0 with at least three repeats for each experiment. Difference between the two groups was compared by
Student’s t-test, and difference among more than two groups was analyzed via ANOVA followed by Tukey’s post hoc test. Pearson correlation analysis was used to analyze the interaction relationship between variables. $P<0.05$ was considered as statistically significant.

**Results**

**MALAT1 is Enhanced in DDP-Resistant OC Tissues and Cells**

qRT-PCR assay demonstrated that MALAT1 expression was upregulated in DDP-resistant OC tissues (Figure 1A and Supplement Figure 1A). As exhibited in Figure 1B and Supplement Figure 2A and B, IC$_{50}$ of DDP in resistant cells was apparently elevated compared with SKOV3 and OVCAR3 cells. Then, we detected the level of MALAT1 in DDP-resistant OC cells. As expected, MALAT1 level was remarkably increased in SKOV3/DDP and OVCAR3/DDP cells (Figure 1C). Therefore, MALAT1 might act as an oncogene in DDP-resistant OC development.

**MALAT1 Knockdown Restrains DDP-Resistant OC Progression**

DDP-resistant cells were transfected with si-NC, si-MALAT1#1, si-MALAT1#2 to investigate the function of MALAT1 in DDP-resistant OC. Knockdown efficiency was confirmed by qRT-PCR assay (Figure 2A). As demonstrated in Figure 2B, MALAT1 knockdown dramatically reduced IC$_{50}$ of DDP. Furthermore, MTT assay suggested that cell proliferation was suppressed by MALAT1 depletion in SKOV3/DDP and OVCAR3/DDP cells (Figure 2C and D, Supplement Figure 2C and D, and Supplement Figure 3A and B). In addition, proportion of cell apoptosis was detected using flow cytometry. As shown in Figure 2E and F and Supplement Figure 3C and D, downregulation of MALAT1 significantly facilitated cell apoptosis. Besides, the decreased cell migratory and invasive abilities were observed in MALAT1-depleted SKOV3/DDP and OVCAR3/DDP cells (Figure 2G–J and Supplement Figure 3E–H). These data indicated that MALAT1 knockdown increased DDP resistance, repressed cell proliferation, migration, invasion, and promoted cell apoptosis in DDP-resistant OC cells.

**MiR-1271-5p is a Target of MALAT1**

Online tool starbase3.0 showed that miR-1271-5p contained the binding sites for MALAT1 (Figure 3A) and dual-luciferase reporter assay verify this prediction. MiR-1271-5p significantly reduced the luciferase activity of MALAT1-WT, not MALAT1-MUT, in SKOV3/DDP and OVCAR3/DDP cells, revealing that MALAT1 interacted with miR-1271-5p (Figure 3B and C). Moreover, the results manifested that MALAT1 knockdown accelerated miR-1271-5p expression in SKOV3/DDP and OVCAR3/DDP cells (Figure 3D). Besides, miR-1271-5p level was significantly downregulated in DDP-resistant OC tissues and cells (Figure 3E, Supplement Figure 1A and Figure 3F). Furthermore, qRT-PCR assay confirmed that miR-1271-5p expression was negatively correlated with MALAT1 expression in DDP-resistant OC tissues (Figure 3G). Therefore, MALAT1 could directly weakened miR-1271-5p expression.

**MiR-1271-5p Overexpression Represses the Proliferation, Migration, and Invasion, but Induces Apoptosis in DDP-Resistant OC Cells**

QRT-PCR assay verified that transfection with miR-1271-5p remarkably increased miR-1271-5p expression (Figure 4A).
The results demonstrated that IC₅₀ of DDP was significantly reduced by miR-1271-5p overexpression (Figure 4B and Supplement Figure 2E and F). Next, we found that miR-1271-5p overexpression significantly suppressed cell proliferation in SKOV3/DDP and OVCAR3/DDP cells (Figure 4C and D). Furthermore, flow cytometry was carried out to...
Figure 3 The prediction and confirmation of the interaction between MALAT1 and miR-1271-5p. (A) The interaction between MALAT1 and miR-1271-5p was predicted by starbase3.0. The red color represented the mutant binding sites. (B and C) The luciferase activity was determined in SKOV3/DDP and OVCAR3/DDP cells (n=3). (D) MiR-1271-5p expression was analyzed by qRT-PCR assay (n=3). (E) MiR-1271-5p expression was detected in DDP-resistant (n=25) and DDP-responsive OC tissues (n=34). (F) MiR-1271-5p expression was detected in DDP-resistant and DDP-responsive OC cells (n=3). (G) The relationship between miR-1271-5p expression and MALAT1 expression (n=3). *P<0.05.

detect apoptotic cells. As indicated in Figure 4E, miR-1271-5p upregulation dramatically induced cell apoptosis. Besides, cell migratory and invasive abilities were lower in miR-1271-5p-upregulated SKOV3/DDP and OVCAR3/DDP cells than that in control cells (Figure 4F and G).

MiR-1271-5p Targets E2F5 and Suppresses E2F5 Expression

Online tool targetscan demonstrated that E2F5 3’UTR contained a complementary sequence with miR-1271-5p (Figure 5A). The results showed that the luciferase activity of E2F5-WT, but not E2F5-MUT, was reduced by miR-1271-5p (Figure 5B and C). Figure 5D and E demonstrated that the mRNA and protein level of E2F5 was downregulated by overexpression of miR-1271-5p. Besides, increased E2F5 expression was observed in DDP-resistant OC tissues and cells (Figure 5F–I and Supplement Figure 1C). Furthermore, we found that E2F5 expression was negatively correlated with miR-1271-5p expression in DDP-resistant OC tissues (Figure 5J). These data uncovered that miR-1271-5p inversely regulated E2F5 expression.

MALAT1 Impedes miR-1271-5p Expression to Upregulate E2F5

Our data showed that there was a positive association between E2F5 expression and MALAT1 expression in DDP-resistant OC tissues (Figure 6A). Next, si-MALAT1#1 + anti-miR-1271-5p, si-MALAT1#1 + anti-miR-NC, si-MALAT1#1, si-NC, miR-1271-5p + pcDNA, or miR-1271-5p + E2F5 was transfected into SKOV3/DDP and OVCAR3/DDP cells. QRT-PCR assay and Western blot assay confirmed that E2F5 level was downregulated by MALAT1 knockdown, and then partly restored by miR-1271-5p silencing (Figure 6B and C). Moreover, we confirmed that overexpression of E2F5 elevated E2F5 level under miR-1271-5p overexpression condition (Figure 6B and C). Overall, MALAT1 upregulated E2F5 level via targeting miR-1271-5p.

MALAT1 Regulates DDP-Resistant OC Cell Progression Through Modulating miR-1271-5p/E2F5 Axis

As shown in Figure 7A, IC50 of DDP downregulated by MALAT1 knockdown was increased by miR-1271-5p depletion. MALAT1 downregulation-inhibited cell proliferation was promoted by miR-1271-5p depletion (Figure 7B and C). Furthermore, we found that the promotion effect of MALAT1 knockdown on cell apoptosis was reversed by miR-1271-5p depletion in SKOV3/DDP and OVCAR3/DDP cells (Figure 7D). Besides, MALAT1 knockdown repressed cell migration and invasion, which
were blocked by depletion of miR-1271-5p (Figure 7E and F). The above results confirmed that miR-1271-5p overexpression repressed the growth of SKOV3/DDP and OVCAR3/DDP cells. Taken together, MALAT1 regulated the development of DDP-resistant OC cells by modulating miR-1271-5p/E2F5 axis.

**Discussion**

In recent years, DDP is an important drug for the therapy of OC, while OC cells prone to generate drug resistance in OC treatment. Moreover, lncRNAs were proved to be associated with DDP resistance in OC. For example, Long et al confirmed that lncRNA GAS5 increased DDP-resistance and...
promoted OC cell growth through modulating MAPK pathway. Miao et al reported that lncRNA ANRIL changed the sensitivity of OC cells to DDP by mediating let-7a and HMGA2 levels. LncRNA linc00312 regulated DDP resistance of OC cells via modulating Bcl-2/Caspase-3 pathway. Therefore, lncRNAs exerted the crucial function in DDP-resistant OC cells.

MALAT1, as an oncogene, was highly expressed in tumors, such as hepatocellular carcinoma, breast cancer, gastric cancer, and colorectal cancer. In this study, increased MALAT1 expression was discovered in DDP-resistant OC tissues and cells. Moreover, MALAT1 knockdown decreased DDP resistance, suppressed proliferation, mobility, and promoted apoptosis of DDP-resistant OC cells. Our results were consistent with the previous data. For example, Bai and his colleagues showed that MALAT1 level was upregulated, while MALAT1 depletion inhibited cell development and DDP resistance in OC tissues or DDP-resistant OC tissues. These data revealed that MALAT1 played a pivotal role in the development of DDP-resistant OC cells.

LncRNAs act as ceRNAs to regulate the levels of downstream miRNAs through binding to target genes. For instance, lncRNA WDFY3-AS2 targeted miR-18a and repressed miR-18a expression. In this study, we observed that miR-1271-5p likely interacted with MALAT1. Subsequently, the relationship between miR-1271-5p and MALAT1 was confirmed by dual-luciferase reporter assessment. Moreover, we confirmed that MALAT1 inversely mediating miR-1271-5p level in DDP-resistant OC cells.

**Figure 5** The prediction and confirmation of the interaction between miR-1271-5p and E2F5. (A) The binding sites of miR-1271-5p and E2F5. (B and C) The luciferase activity was determined (n=3). (D and E) The mRNA and protein level of E2F5 were detected by qRT-PCR and Western blot in SKOV3/DDP and OVCAR3/DDP cells transfected with miR-NC or miR-1271-5p (n=3). (F-I) The mRNA and protein level of E2F5 were detected in DDP-resistant and DDP-responsive OC tissues (F and G) and cells (H and I). (J) The association between miR-1271-5p and E2F5 expression was detected (n=3). *P<0.05.
A previous research showed that miR-1271 level was upregulated, and miR-1271 upregulation attenuated cell proliferation but enhanced apoptosis in DDP-resistant OC cells. \(^\text{15}\) In agreement with these data, our results confirmed that miR-1271-5p upregulation repressed the growth and DDP resistance of DDP-resistant OC cells. Previously, accumulating evidence demonstrated that miR-1271-5p impeded the development in human cancers. Yang and his colleagues indicated that miR-1271-5p, downregulated by lncRNA UCA1, suppressed cell proliferation, and enhanced apoptosis in multiple myeloma. \(^\text{35}\) Additionally, miR-1271-5p repressed the growth of hepatocellular carcinoma cells via modulating FOXK2 expression. \(^\text{36}\) Thus, we hypothesized that MALAT1 mediated DDP-resistant OC cell progression through inhibiting miR-1271-5p expression. Our data revealed that miR-1271-5p knockdown restored the effect of MALAT1 depletion on DDP-resistant OC cell progression.

Next, online tool targetscan predicted E2F5 was a potential downstream gene of miR-1271-5p and our results confirmed that miR-1271-5p could bind to E2F5. Moreover, E2F5 level was downregulated by miR-1271-5p. Previous evidence suggested that E2F5 expression was enhanced in OC tissues, and E2F5 acted as a positive regulator in OC cell growth. \(^\text{22}\) However, there was no reporter about the function of E2F5 in DDP-induced OC cells. In this paper, we discovered that E2F5 was highly expressed in DDP-resistant OC cells, and E2F5 overexpression promoted DDP-resistant OC cell growth that suppressed by miR-1271-5p upregulation. Thus, we concluded that miR-1271-5p repressed E2F5 expression to modulate the growth of DDP-resistant OC cells.
In human cancers, lncRNA can regulate the levels of multiple miRNAs, and miRNA can control various coding genes.\(^{37,38}\) Thus, it is speculated that there are other genes mediated by MALAT1 and miR-1271-5p in DDP-resistant OC cells. Therefore, more experiments were needed to understand the mechanisms of MALAT1 and miR-1271-5p in DDP-resistant OC cells.

Figure 7 The MALAT1/miR-1271-5p/E2F5 axis in the progression of DDP-resistant OC cells. (A) IC\(_{50}\) of cisplatin was examined (n=3). (B and C) Measurement of cell proliferation by MTT assay (n=3). (D) Determination of cell apoptosis by flow cytometry (n=3). (D-F) Cell migratory and invasive abilities were determined by transwell assays (magnification × 100) (n=3). *P<0.05.
In conclusion, we demonstrated that MALAT1 knockdown suppressed DDP resistance, proliferation, and mobility, and promoted apoptosis by miR-1271-5p/E2F5 axis in DDP-resistant OC cells, providing a novel target for the therapy of OC.

**Highlights**

1. The relationship between MALAT1 and miR-1271-5p is confirmed for the first time.
2. The relationship between miR-1271-5p and E2F5 is confirmed for the first time.
3. MALAT1 knockdown represses IC50 of cisplatin and growth of DDP-resistant OC cells.
4. MALAT1 regulates the development of DDP-resistant OC cells through modulating miR-1271-5p/E2F5 axis.

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**Disclosure**

The authors declare that they have no financial or non-financial conflicts of interest for this work.

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