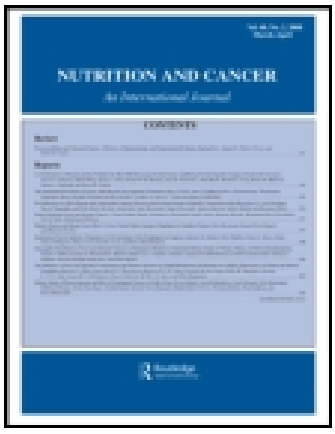

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EGCG Enhances the Efficacy of Cisplatin by Downregulating hsa-miR-98-5p in NSCLC A549 Cells

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In the current study, the enhanced efficacy of cisplatin caused by (-)-epigallocatechin-3-gallate (EGCG) in nonsmall cell lung cancer (NSCLC) A549 cells was observed. The tumor size was significantly smaller in vivo in the combination of cisplatin and EGCG group, as compared with cisplatin-only group. However, in NCI-H460 cells, another kind of NSCLC cells, the efficacy of cisplatin was antagonized by EGCG. MiRNA microarray showed that hsa-miR-98–5p and hsa-miR-125a-3p were differentially expressed after EGCG treatment in these 2 cell lines. After transfection of hsa-miR-98–5p inhibitor, the survival fraction of both A549 and NCI-H460 cells was decreased upon cisplatin treatment. Meanwhile, as a critical regulator in the cisplatin-induced apoptosis, p53 was elevated by silencing of hsa-miR-98–5p. These results suggested that EGCG inhibited the expression of hsa-miR-98–5p, followed by an increase of p53, thus the efficacy of cisplatin was enhanced. Bioinformatics analysis showed that hsa-miR-125a-3p might have a strong connection with classical MAPK pathway. Taken together, these findings indicate that hsa-miR-98–5p could be a potential target in clinical cisplatin treatment of NSCLC. The combination of EGCG and cisplatin might be an effective therapeutic strategy in treating some type of NSCLC, although the possibility of antagonistic interactions must also be taken into account.

INTRODUCTION

Lung cancer is a leading cause of cancer death worldwide (1). Although in the recent decades, new therapeutic strategies are under investigation, chemotherapy still plays an important role in the treatment of lung cancer, especially nonsmall cell lung cancer (NSCLC) (1). *Cis*-diamminedichloroplatinum(II) (best known as cisplatin or cDDP) is the first line drug for NSCLC chemotherapy. The main antitumor mechanism involves causing DNA damage, forming DNA-Pt complex and thus inducing apoptosis (2). Once DNA damage happens, the expression of p53 is elevated and attempted to repair the damage. If that doesn't work, the damaged cells will be induced to the p53-depended apoptosis (2).

Cisplatin exerts clinical activity against a wide spectrum of solid neoplasm (3) and often leads to an initial therapeutic success. However, many patients are intrinsically resistant to cisplatin-based therapies, and some originally sensitive tumors eventually develop chemoresistance (4). Besides, the cytotoxicity of cisplatin also affects kidney, peripheral nerves, and the inner ear (5). All these are the limitations to the clinical application of cisplatin.

Considering the poor prognosis and the serious toxic and side effects of cisplatin, scientists are now trying to explore new combination chemotherapies, so as to achieve higher efficacy and to avoid the serious side effects. (-)-Epigallocatechin-3-gallate (EGCG) is the major catechin found in green tea and has been intensively studied as a chemopreventive agent (6,7). It has shown anticancer effect (including prostate, head and neck, and lung cancer) in many in vitro and in vivo studies (8–10). In addition to the antitumor activity of EGCG itself, it is reported that EGCG could enhance the efficacy of several chemotherapy drugs, as well as antagonize their toxicity (11,12).

Several studies had investigated the combination of EGCG and cisplatin. EGCG could promote the cellular accumulations of platinum and DNA-Pt binding, and the following apoptosis (13). EGCG also significantly inhibited telomerase expression, enhanced the effect of cisplatin, and made the tumor cells more sensitive to the treatment (14). In 8 ovarian cancer cell lines, EGCG treatment led to an enhanced intracellular H_2O_2 generation, suggesting that EGCG might accentuate oxidative stress to sensitize ovarian cancer cells to cisplatin (15). On the other hand, EGCG could protect the tissues and organs from nephrotoxicity, neurotoxicity, and ototoxicity of cisplatin (16–18).

MicroRNA (miRNA) becomes a new research hotspot in the recent decade. MiRNAs are short noncoding RNAs of 20–24 nucleotides that involve in the regulation of several key cellular processes (19,20). In the cytoplasm, mature miRNA binds to the 3'untranslated regions (3'UTRs) of target mRNAs,

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incorporating into the RNA-induced silencing complex (RISC), then induces posttranscriptional gene silencing (20).

A single miRNA can target multiple genes. They act as regulators of gene expression and protein synthesis, and play important roles in cell growth, differentiation, metabolism and apoptosis (21,22). Recently, a number of miRNAs have been linked to different human cancers, they could affect the progression of tumors through regulation of key cancer-related signal pathways, cell cycle arrest and DNA damage response (20). Their roles in cell proliferation and apoptosis have drawn special attention in anticancer research. So far, miRNAs have already entered the clinic as diagnostic and prognostic biomarkers for patient stratification and also as therapeutic targets (20).

Although former studies have demonstrated that EGCG could regulate certain miRNAs, inducing apoptosis or suppressing cell growth in several human cancer cells (23,24), there is still no research focuses on the miRNAs, through which EGCG may enhance the efficacy of cisplatin. In the current study, we investigated whether EGCG could affect the efficacy of cisplatin in NSCLC A549 cells, and assuming that this phenomenon was owing to the regulation of the certain miRNAs by EGCG pretreatment. Our results suggested that hsa-miR-98–5p might be a potential target in clinical cisplatin treatment of NSCLC. Also, we provided preliminary evidence on EGCG-cisplatin combination therapy of NSCLC in clinical research.

MATERIALS AND METHODS

Cell Culture and Reagents

Human nonsmall cell lung cancer A549, NCI-H460, and LTEP-α-2 cells were purchased from Cell Centre of Chinese Academy of Medical Sciences (Beijing, China) and were maintained in Dulbecco's Modified Eagle Medium (DMEM, GIBCO, Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO, Gaithersburg, MD) and 1% penicillin/streptomycin (Beyotime Institute of Biotechnology, Shanghai, China). Cells were incubated in a humidified atmosphere of 5% CO_2 at 37°C. EGCG and cisplatin were purchased from Sigma-Aldrich (St. Louis, MO).

MTT Assay

Cell surviving fraction was detected by MTT assay. Cells (2×10^3 per well) were seeded in a 96-well plate with DMEM containing 1% FBS and allowed to attach for 24 h. Then EGCG and cisplatin were added alone or in combination. After 24 h treatment, cells were incubated with 20 μL of MTT solution (5 mg/mL, AMRESCO, Solon, OH) for 4 h at 37°C. The MTT formazan crystal was then dissolved in 150 μL DMSO (Lingfeng, Shanghai, China), and the absorbance was measured at 490 nm using a microplate reader (TECAN, Männedorf, Switzerland).

Xenograft Mouse Model

Institutional approval was acquired from the Ethical Review Board of Nanjing Medical University prior to this study. Female BALB/c nude mice with 3–4 wk old were purchased from Slac Laboratory Animal (Shanghai, China). A549 cells (5×10^6) were injected into the dorsal of the mice. The body weight and the tumor size were recorded every day. Tumor size was measured using a caliper, and the volumes were calculated using the following formula: volume (mm^3) = length × width × width/2. Once the average volume reached $100mm^3$, 18 mice were randomized into 3 groups (6 mice/group) as follows: control (NS, 0.1 ml/10 g), cisplatin (5 mg/kg), and EGCG (20 mg/kg) combined with cisplatin (5 mg/kg). All the agents were administered twice a week for 2 wk through intraperitoneal injection. EGCG was given 1 day before cisplatin. Another 6 mice were given EGCG (20 mg/kg) alone every 3 days through intraperitoneal injection. All animal experiments were performed according to procedures approved by The Center for Hygienic Analysis and Detection of Nanjing Medical University.

MiRNA Microarray Analysis

After the EGCG (10 μmol/L) treatment for 24 h, total RNA of the cells was extracted and prepared by RNAiso Plus (TaKaRa BioTechnology, Dalian, Liaoning, China). Then the prepared RNA was sent to CT Bioscience (Changzhou, Jiangsu, China) for miRNA microarray analysis. Ten differentially expressed miRNAs ($\geq$2.5-fold, or which had different responds to EGCG in A549 and NCI-H460 cells) before and after EGCG treatment were selected.

Bioinformatics Analysis

The bioinformatics analysis of hsa-miR-125–3p was performed by RiboBio Co., Ltd. (Guangzhou, Guangdong, China).

Real-Time Polymerase Chain Reaction

Total RNA was extracted by the RNAiso Plus (TaKaRa BioTechnology, Dalian, Liaoning, China) following the manufacturer's protocol. For miRNA quantitative analysis, total RNA was reverse transcribed using the PrimeScript™ RT Master Mix (TaKaRa BioTechnology), and qPCR was performed using SYBR® Premix Ex Taq™II (TaKaRa BioTechnology). The miRNA primers were purchased from RiboBio Co., Ltd. (Guangzhou, Guangdong, China). All qPCR was performed with the Applied Biosystems 7300 Real Time PCR System (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Expression of miRNA was defined from the threshold cycle, and relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method after normalization with reference to the expression of U6 small nuclear RNA.

Reverse-Transcriptase PCR

Total RNA was extracted by the RNAiso Plus (TaKaRa BioTechnology) following the manufacturer's protocol. Total RNA was reverse transcribed using the PrimeScript™ RT

Master Mix (TaKaRa Bio Technology), and PCR was performed using Premix Taq™ (TaKaRa Taq™ Version 2.0 plus dye, TaKaRa BioTechnology) under the following conditions: denaturation for 30 s at 95°C followed by 30 cycles of 15 s at 95°C, 30 s at 55°C, and 30 s at 72°C, with extension for 10 min at 72°C. The mRNA specific primers were purchased from Genscript Corp. (Nanjing, Jiangsu, China). The PCR products were separated by electrophoresis in 2% agarose gels and were detected using the AlphaImager HP system (Alpha Innotech, San Leandro, CA).

Transfection of Mimic/Inhibitor of miRNAs

The mimic and inhibitor of hsa-miR-98–5p and hsa-miR-125a-3p were purchased from RiboBio Co., Ltd (Guangzhou, Guangdong, China). The mimic/inhibitor of certain miRNAs was transfected into the cells using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). All steps were followed the manufacturer's instructions.

Statistical Methods

Data were presented as the mean ± SD. All the experiments were performed no less than 3 times. To assess the statistical significance of differences, student's *t*-test or 1-way analysis of variance was performed. The data were considered statistically significant when the *P* value was less than 0.05.

RESULTS

EGCG Enhanced the Efficacy of Cisplatin in Certain NSCLC Cells

The effect of cisplatin, EGCG, and the combination of cisplatin and EGCG on A549 cells growth was investigated. The results showed that cisplatin and EGCG inhibited the growth of A549 cells in a dose-dependent manner (Fig. 1A and 1B). At a lower concentration (<10 μmol/L), EGCG had little impact on the proliferation of A549 cells.

The sequence of administration could affect the ultimate efficacy when EGCG combined with some antitumor drugs (13,25). More MTT assays with different sequences of administration were performed. According to the results, pretreatment of EGCG for 4 h before cisplatin (named 0/4) had the most obvious inhibitory effect in A549 cells (Fig. 1C). Based on this sequence, we observed that EGCG (10 μmol/L) could enhance the efficacy of cisplatin in multiple concentrations (Fig. 1D). The same conclusion was also drawn in LTEP-α-2 cells, which was another kind of NSCLC cells (Fig. 1E).

EGCG Enhanced the Efficacy of Cisplatin In Vivo

Whether EGCG could enhance the efficacy of cisplatin in vivo was further investigated. An A549 xenograft nude mice model was established as described in the **Materials and Methods** section. The tumor size was recorded everyday (Fig. 2A–2C). On the 15th day, all animals were sacrificed. The results showed that EGCG combined with cisplatin had a stronger inhibitory effect on the growth of tumors than cisplatin alone in vivo (Fig. 2D), consistent with the above in vitro experiments.

EGCG Attenuated the Efficacy of Cisplatin in NSCLC NCI-H460 Cells

In another NSCLC NCI-H460 cells, cisplatin killed the cells in a dose-dependent manner (Fig. 3A). To our surprise, EGCG promoted the proliferation of NCI-H460 cells significantly in the low concentrations (10–20 μmol/L) (Fig. 3B). Besides, the efficacy of cisplatin was attenuated by EGCG pretreatment for 4 h (0/4) (Fig. 3C). MTT assays with different sequences of administration were carried out, and the same results were obtained (data not shown).

hsa-miR-98-5p Was Downregulated in A549 Cells While Upregulated in NCI-H460 Cells by EGCG

MiRNAs may have influences on the curative effect of some chemotherapy drugs (26,27). Because EGCG had different influences on NSCLC cells in cisplatin treatment, whether EGCG could affect the efficacy of cisplatin through certain miRNAs was investigated.

Total RNA from EGCG-treated A549 and NCI-H460 cells was isolated respectively. MiRNA microarray analysis was preformed to select 10 differentially expressed miRNAs (≥2.5-fold, or which had different responds to EGCG in A549 and NCI-H460 cells) before and after EGCG treatment (Fig. 4A). The results revealed that hsa-miR-98–5p was downregulated in A549 cells, while upregulated in NCI-H460 cells, consistence with the results from RT-qPCR (Fig. 4B). In the A549 xenograft nude mice models, hsa-miR-98–5p was elevated in tumors compared with the lung tissues, whereas after the EGCG treatment, hsa-miR-98–5p in tumors was decreased, which closed to the levels in normal lung tissues (Fig. 4C).

EGCG Decreased the Expression of hsa-miR-98-5p to Enhance the Efficacy of Cisplatin

Because hsa-miR-98–5p had different responses to EGCG in A549 and NCI-H460 cells, we assumed that EGCG might enhance the efficacy of cisplatin through downregulating hsa-miR-98–5p in A549 cells. To test this hypothesis, A549 and NCI-H460 cell were transfected with mimic or inhibitor of hsa-miR-98–5p (Supplemental Fig. S7A and S7B), the survival fraction of the cells responding to cisplatin was measured. The efficacy of cisplatin was significantly strengthened in both A549 and NCI-H460 cells, in which hsa-miR-98–5p was lowly expressed (Fig. 5A).

Cisplatin could induce p53-depended apoptosis (28), and it was predicted that hsa-miR-98–5p could bind to the 3'UTR of p53 mRNA (29). In the current experiment, hsa-miR-98–5p could inhibit the expression of p53 mRNA (Fig. 5B). As it was reported that EGCG could increase p53 mRNA before (8), we considered that in A549 cells, EGCG downregulated the expression of hsa-miR-98–5p, followed by an increasing of p53, activated the p53-depended apoptosis together with cisplatin, thus, the efficacy of cisplatin was enhanced (Fig. 5C).

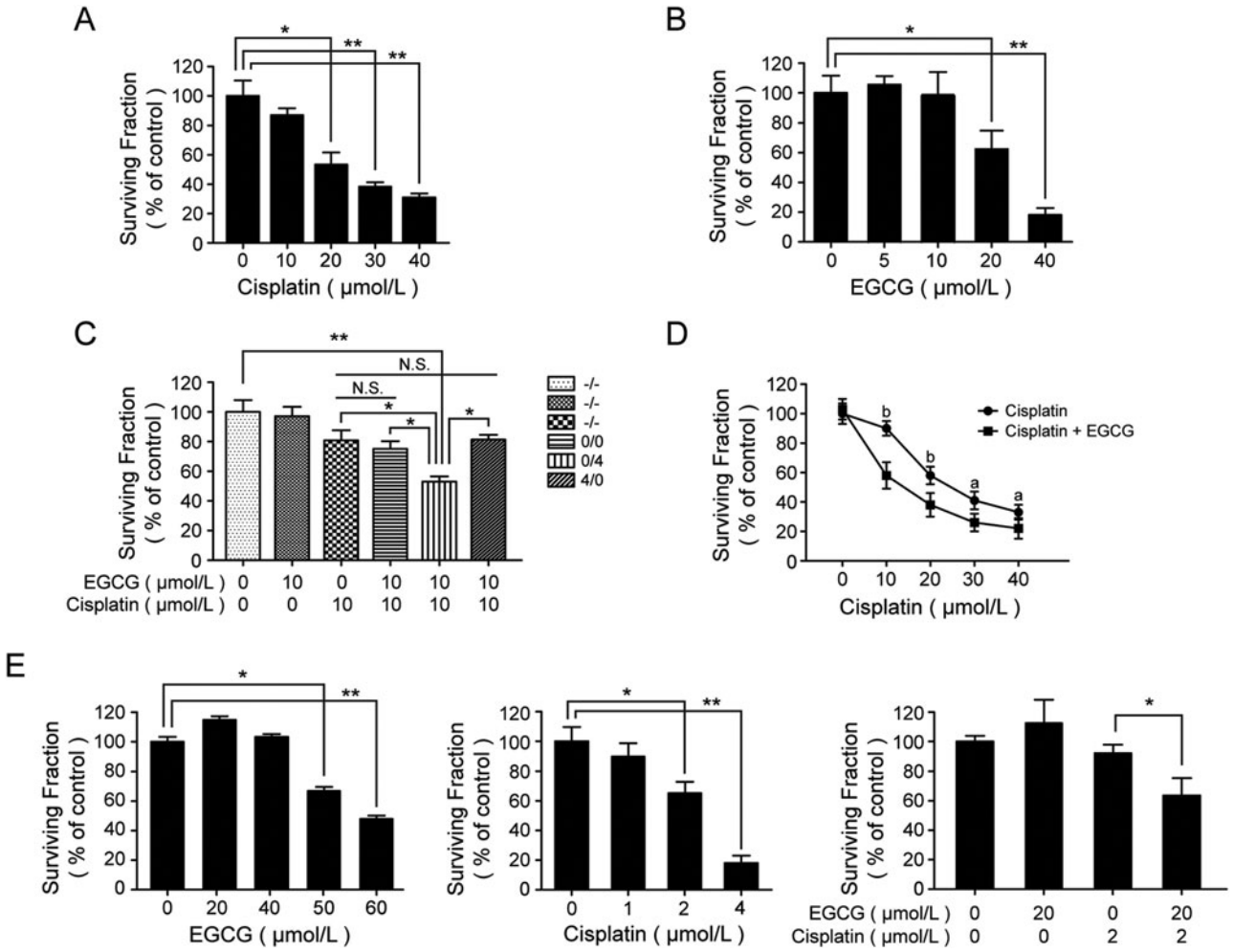

FIG. 1. (-)-Epigallocatechin-3-gallate (EGCG) enhanced the efficacy of cisplatin in certain NSCLC cells. A: The surviving fraction of A549 cells after the cisplatin treatment for 24 h. B: The surviving fraction of A549 cells after the EGCG treatment for 24 h. C: The sequence of administration affected the ultimate surviving fraction. 0/0 = EGCG and cisplatin were administrated simultaneously for 24 h; 0/4 = 24 h of EGCG and cisplatin treatment with 4 h of pretreatment of EGCG; 4/0 = 24 h of EGCG and cisplatin treatment with 4 h of pretreatment of cisplatin. D: EGCG (10 μmol/L) could enhance the efficacy of cisplatin in multiple concentrations. (Compare with the cisplatin treatment, a: $P < 0.05$, b: $P < 0.01$.) E: The surviving fraction of LTEP-α-2 cells after the indicated treatments for 24 h. N.S. = Not Significant. $^{*}P < 0.05$, $^{**}P < 0.01$.

EGCG Could Promote the Proliferation of NCI-H460 Cells Through Decreasing hsa-miR-125a-3p

Another miRNA, hsa-miR-125a-3p, were decreased significantly in NCI-H460 cells by EGCG treatment. Whereas in A549 cells, hsa-miR-125a-3p was slightly suppressed (Figs. 4A and 6A). In the A549 xenograft nude mice model, the expression of hsa-miR-125a-3p had no significant difference among normal lung tissues, tumors of control group, and tumors of EGCG-treated group (Fig. 6B). Low expression of hsa-miR-125a-3p (Supplemental Fig. S7C) promoted the proliferation of both A549 and NCI-H460 cells (Fig. 6C). This could be the reason that the low concentration of EGCG could promote the cells growth (Fig. 1B, 1E, and 3B).

A preliminary bioinformatics analysis was performed to explore the biological function of hsa-miR-125a-3p. Potential target genes were predicted using TargetScan, miRanda, Starbase (Clip-seq), and miRDB database (Fig. 6D and Supplemental Table S1). RAP2B, MEAF6, PPAPDC2, and SEP11 were 4 most probable target genes. KEGG pathway analysis (Table 1 and Supplemental Fig S1–S6) showed that hsa-miR-125a-3p had a strong connection with the classical MAPK signaling pathway (Table 1 and Supplemental Fig. S4), which played an important role in the regulation of cell proliferation.

DISCUSSION

MiRNAs have become important therapeutic targets in several human tumors. It was reported that in many types of cancers, certain special miRNAs had been identified as specific biomarkers for diagnosis and prognosis (19). In the current study, EGCG was found to inhibit the expression of

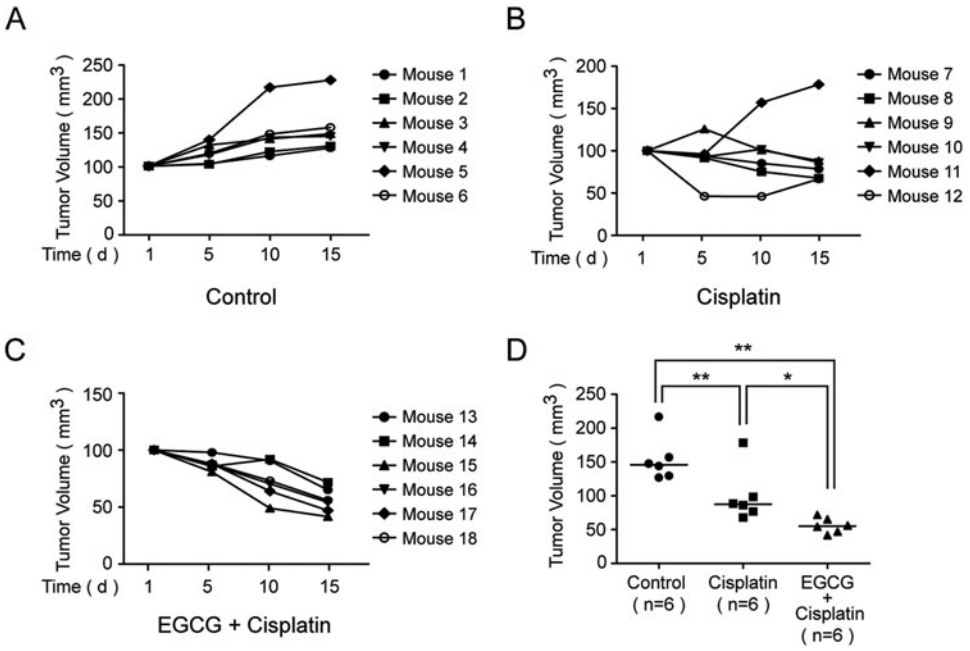

FIG. 2. (-)-Epigallocatechin-3-gallate (EGCG) enhanced the efficacy of cisplatin in vivo. A: Tumor growth curves of each single mouse of control group. B: Tumor growth curves of each single mouse of cisplatin treatment group. C: Tumor growth curves of each single mouse of EGCG combined with cisplatin treatment group. D: The tumor volumes of the mice. $^{*}P < 0.05$, $^{**}P < 0.01$.

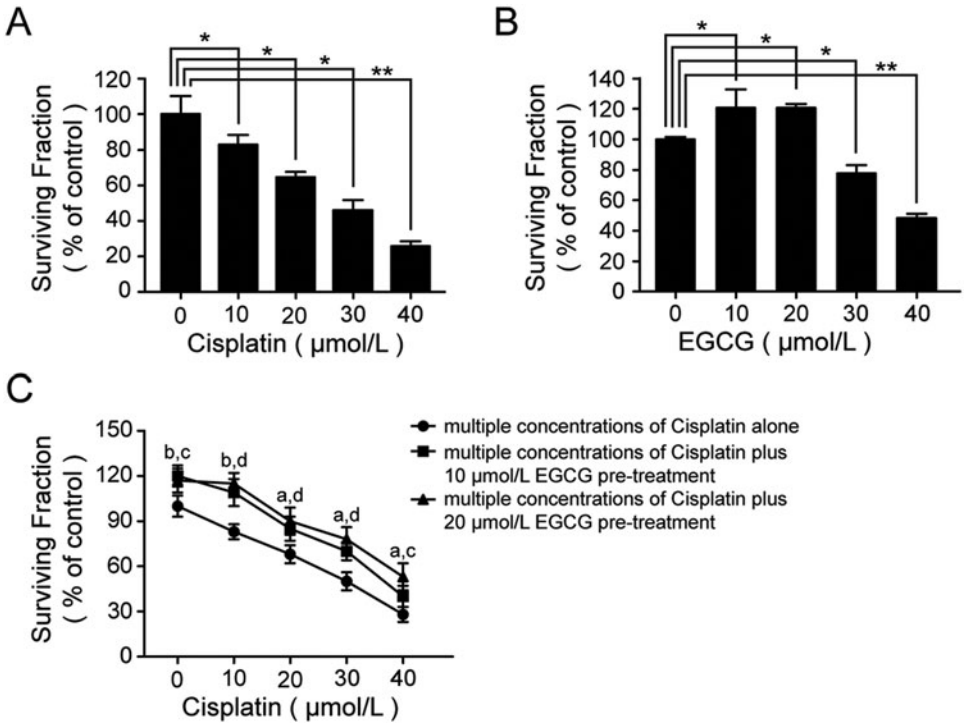

FIG. 3. (-)-Epigallocatechin-3-gallate (EGCG) attenuated the efficacy of cisplatin in NCI-H460 cells. A: The surviving fraction of NCI-H460 cells after the cisplatin treatment for 24 h. B:) The surviving fraction of NCI-H460 cells after the EGCG treatment for 24 h. $^{*}P < 0.05$, $^{**}P < 0.01$. C: EGCG could attenuate the efficacy of cisplatin in multiple concentrations. (The cisplatin + 10 μmol/L EGCG group compared with the cisplatin group, a: $P < 0.05$; b: $P < 0.01$; the cisplatin + 20 μmol/L EGCG group compared with the cisplatin group; c: $P < 0.05$, d: $P < 0.01$.)

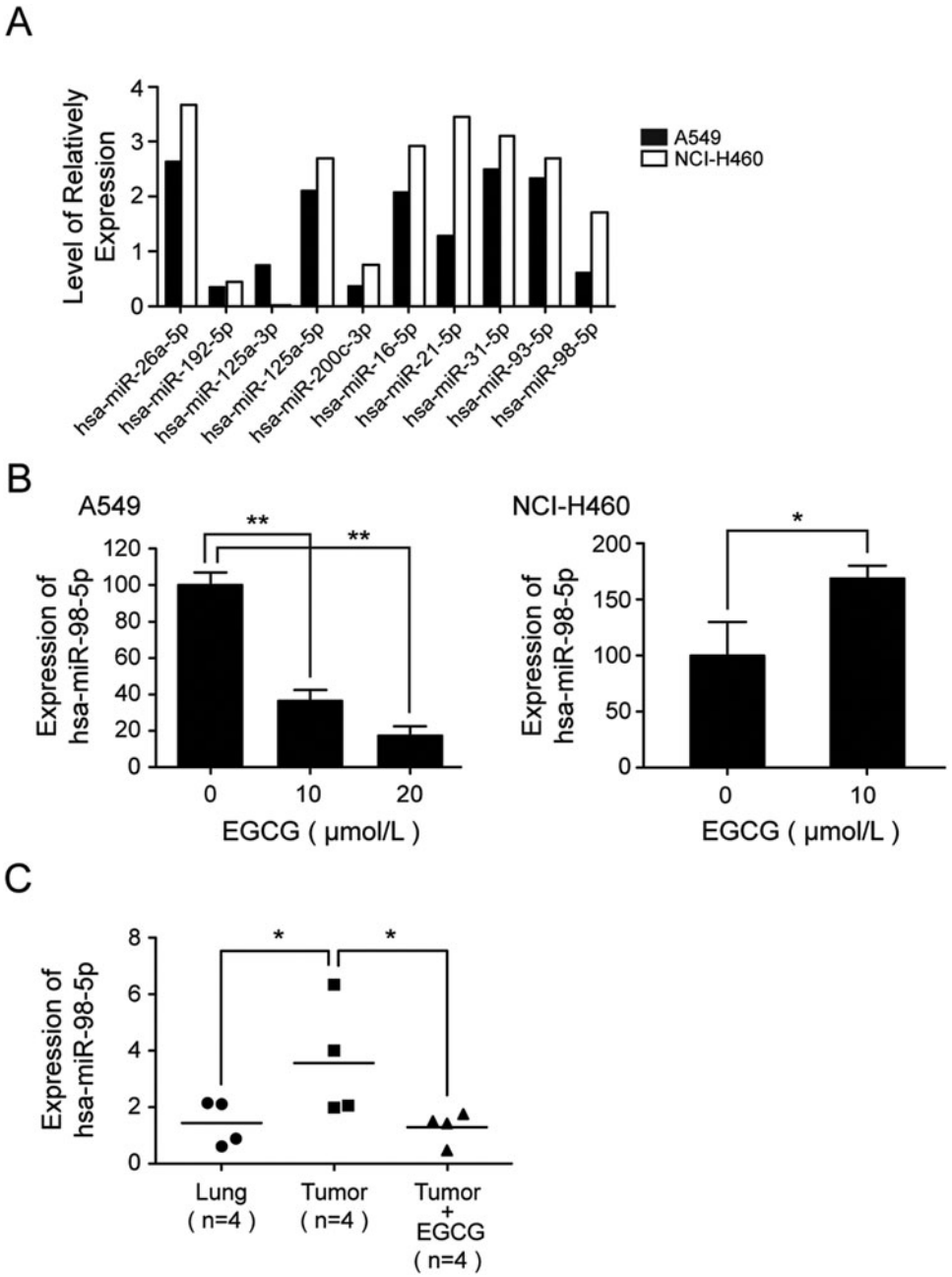

FIG. 4. hsa-miR-98–5p was downregulated in A549 cells while upregulated in NCI-H460 cells by (-)-Epigallocatechin-3-gallate (EGCG). A: Ten differentially expressed miRNAs (≥2.5-fold, or which had different responds to EGCG in A549 and NCI-H460 cells) were selected by miRNA array analysis. B: The expression of hsa-miR-98–5p in A549 and NCI-H460 cells by RT-qPCR. C: The expression of hsa-miR-98–5p in the A549 xenograft nude mice models. $^{*}P < 0.05$, $^{**}P < 0.01$.

hsa-miR-98–5p, followed by an increase of p53 in A549 cells. Thus, the inhibition of cell growth by cisplatin was enhanced (Fig. 5C). It was interesting that hsa-miR-98-5p was promoted by EGCG in NCI-H460 cells, which led to an attenuation of the curative effect of cisplatin. By silencinghsa-miR-98–5p, an enhancement of the efficacy of cisplatin was observed in NCI-H460 cells. Although more detailed molecular mechanisms need to be investigated, to our knowledge, this is the first research reporting that EGCG could enhance the efficacy of cisplatin through downregulation of hsa-miR-98-5p in NSCLC A549 cells, which suggested that hsa-miR-98-5p might be a potential target in clinical cisplatin treatment of NSCLC.

MiRNAs have influences on the curative effect of some antitumor drugs. In several human cancers, scientists are engaged in establishing miRNA expression profiles which associated with the chemosensitivity of the tumor cells (26,27). Recently,

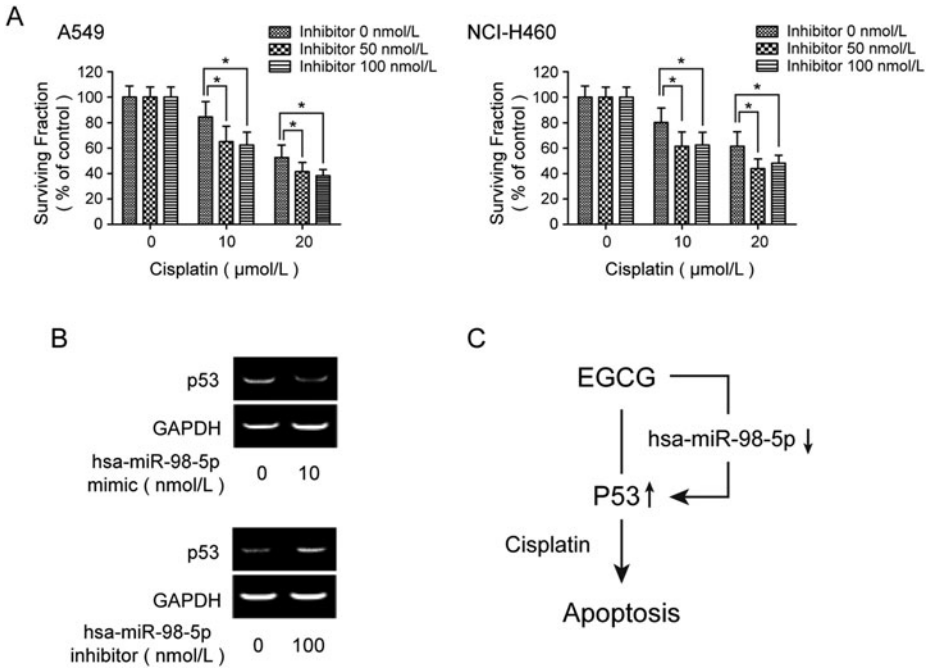

FIG. 5. (-)-Epigallocatechin-3-gallate (EGCG) decreased the expression of hsa-miR-98–5p to enhance the efficacy of cisplatin. A: The efficacy of cisplatin was enhanced after the inhibition of hsa-miR-98–5p in A549 and NCI-H460 cells. B: Hsa-miR-98–5p could regulate p53 mRNA negatively. C: The potential mechanism through which EGCG interacted with cisplatin. $^{*}P < 0.05$, $^{**}P < 0.01$.

the investigation of miRNA-targeted therapies had been applied in clinic successfully (30), which was a tremendous advance from bench to bedside. Because hsa-miR-98-5p could negatively regulate p53, the expression of p53 was elevated in the EGCG pretreated cells. The increased p53 helped to activate the p53-depended apoptosis, thus the curative effect of cisplatin was enhanced. According to several studies, hsa-miR-98-5p was suppressed in the cisplatin-treated cells, indicating that hsa-miR-98-5p has a strong connection with the efficacy of cisplatin (29). We speculate that during the cisplatin

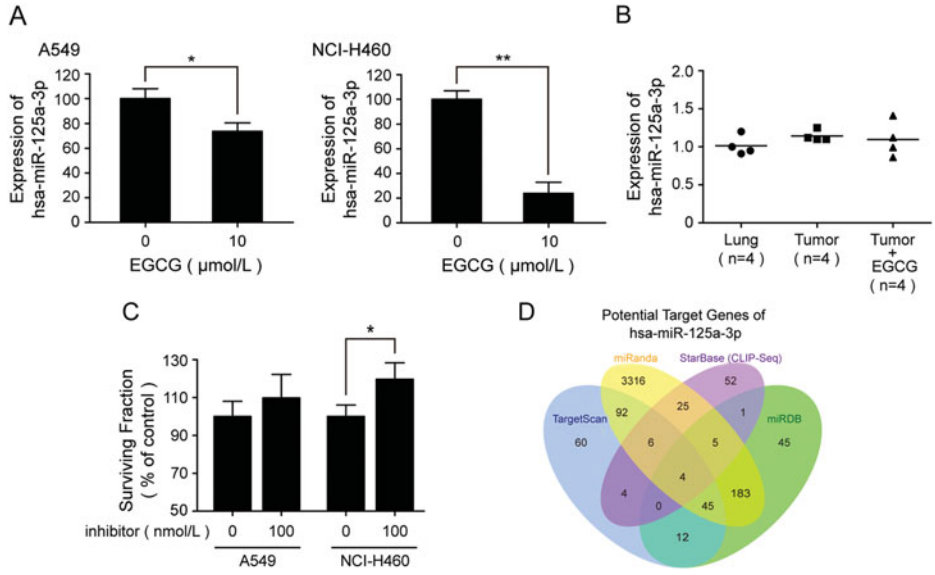

FIG. 6. (-)-Epigallocatechin-3-gallate (EGCG) could decrease the expression of hsa-miR-125a-3p, which might have a strong connection with the proliferation of the cells. A: The expression of hsa-miR-125a-3p in A549 and NCI-H460 cells by RT-qPCR. B: The expression of hsa-miR-125a-3p in the A549 xenograft nude mice models. C: The inhibition of hsa-miR-125a-3p promoted the proliferation of A549 and NCI-H460 cells. D: The potential target genes predicted by 4 databases. $^{*}P < 0.05$, $^{**}P < 0.01$. (Color figure available online.)

TABLE 1
The 6 most relevant pathways predicted by KEGG pathway analysis

Pathway	Pathway ID	Number of possible target genes	*P* value	Gene ID
Pertussis	hsa05133	4	0.00	2771|5601|3586|5602
Dopaminergic synapse	hsa04728	5	0.01	2771|6570|5601|5500|5602
Progesterone-mediated oocyte maturation	hsa04914	4	0.01	2771|6197|5601|5602
MAPK signaling pathway	hsa04010	7	0.01	51701|3554|4763|6197|5601|5602|7786
Retrograde endocannabinoid signaling	hsa04723	4	0.01	2771|5601|2568|5602
Chagas disease (American trypanosomiasis)	hsa05142	4	0.01	2771|5601|3586|5602

therapy, by silencing of hsa-miR-98-p, higher curative effect should be achieved than using cisplatin alone, although lots of further studies are still needed on this subject.

Interestingly, we also noticed that low concentrations of EGCG promoted the cell growth in both A549 and NCI-H460 cells. In fact, a few studies also obtained the proproliferation effect of EGCG in low concentrations, but without any intensive discussions (31,32). We considered that it was because of the inhibition of classical MAPK pathway by hsa-miR-125a-3p. EGCG inhibited the expression of hsa-miR-125a-3p, and bioinformatics analysis showed that this miRNA had a strong connection with the classical MAPK signaling pathway, which could regulate the cell growth, suggesting that hsa-miR-125a-3p might affect the proliferation of the cells. In human gastric cancers, low expression levels of hsa-miR-125a-3p were associated with enhanced malignant potential (33), and hsa-miR-125a-3p also functioned as a tumor suppressor gene that inhibited the cell proliferation, migration, and invasion in lung cancer A549 and SPC-A-1 cells (34,35) and HEK 293T cells (36). Here, we predicted that oncogene RAP2B could be a new target gene of hsa-miR-125a-3p in NSCLC cells, and the proproliferation of EGCG should be drawn more attention in the further researches.

Nowadays, the development of new cancer therapy strategies, including combination of conventional anticancer drugs with bioactive dietary compounds such as EGCG, has gained considerable interest. These new strategies had exerted synergistic activities and decreased therapy-induced toxicity during the chemotherapy. Besides cisplatin, it was reported that EGCG could enhance the efficacy of several chemotherapy drugs. EGCG might enhance the retention of antitumor drugs to synergistically inhibit tumor growth and eradicate tumors (37,38). In several human cancer cells, EGCG could induce cell cycle arrest or apoptosis (11) and inhibit the expression of multidrug resistance proteins (38). As for hormone-related cancers, such as breast and prostate cancer, EGCG interacted with the hormone receptors, delayed the development of hormone refractory tumors, and induced chemosensitization of breast and prostate cancer in vitro and in vivo (39,40). Also, EGCG could act as a protective agent against the side effects of chemotherapy, such as gastrointestinal disorders, declination of hematological parameters and immune system, development of secondary tumors, nephrotoxicity, cardiotoxicity, ototoxicity, and dysfunction of salivary glands and pulmonary fibrosis (12).

Further researches are urgently needed on EGCG-drug combination therapies. In this project, we noticed that in NCI-H460 cells, EGCG failed to decrease hsa-miR-98-5p and the efficacy of cisplatin was attenuated. Our result is not the only case that the curative effect of antitumor drugs been antagonized by EGCG. Low concentrations of EGCG had no effect on bortezomib activity in a xenograft mouse model of prostate cancer, and higher concentrations of EGCG antagonized the anti-tumor effect of bortezomib (41). In another report, reduced bioavailability of the antitumor drug sunitinib has been related to coadministration with EGCG (42). Besides, we also noticed that the low concentrations of EGCG could inhibit hsa-miR-125a-3p, causing a promoted proliferation in both A549 and NCI-H460 cells. All these observations show that EGCG could be a potential adjuvant for cancer therapy in clinic, but further work needs to conduct more studies to ascertain the interaction between EGCG and different antitumor drugs, for each particular type and stage of cancer. Importantly, the possibility of antagonistic interactions must be taken into account in the research of EGCG and antitumor drug combination therapies.

Overall, our research demonstrated that EGCG could enhance the curative effect of cisplatin through downregulation of hsa-miR-98–5p, suggesting that hsa-miR-98–5p might be a potential target in clinical cisplatin treatment of NSCLC. Also, we provided preliminary evidence on EGCG-cisplatin combination therapy of NSCLC in clinical research, however, a lot more comprehensive researches are urgently needed in this subject.

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SUPPLEMENTAL DATA

Supplemental data for this article can be accessed on the publisher's website.

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