Cellular and Molecular Mechanism of Cell Proliferation in Human Gastric Cancer Drug-Resistant Cells After Hyperthermia and Cisplatin: Role of mRNAs and Long-Non-coding RNAs

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ABSTRACT

Background: *Since thermo-chemotherapy was suggested as an effective treatment for gastric cancer, we aimed to evaluate the effects of hyperthermia combined with cisplatin (DDP) on the inhibition of human gastric cancer drug-resistant cells in vitro and explore its possible mechanisms.*

Methods: *SGC-7901/DDP cells were cultured and divided into control, cisplatin, hyperthermia, and hyperthermia combined with cisplatin groups. Hyperthermia was done at 42°C, 44°C, 46°C, 48°C, and 50°C for 12 h, 24 h, 36 h; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay detected the proliferation of SGC-7901/DDP at different time and temperature, and the apoptotic rate of SGC-7901/DDP cells was evaluated by using Annexin staining assay. High-throughput Chromatin immunoprecipitation (ChIP)-seq was applied to test long non-coding RNA expression in SGC-7901/DDP cells. Then, real-time fluorescence quantitative polymerase chain reaction was used to verify the expression of long non-coding RNA in all groups.*

Results: *Double staining showed that hyperthermia combined with cisplatin increased the rate of early apoptosis of SGC-7901/DDP cells. Long non-coding RNA high-throughput ChIP-seq showed a significantly larger amount of long non-coding RNAs and mRNAs in the cells treated with hyperthermia combined cisplatin group in comparison with the control group. We observed that the upregulated mRNAs and long non-coding RNAs were highly related to immune system response and CD95 signaling pathway in nucleus, and down-regulated mRNAs and long non-coding RNA were highly related to Mammalian target of rapamycin (mTOR) and Tumor necrosis factor (TNF) receptor signaling pathway in cytoplasm.*

Conclusion: *Hyperthermia combined with cisplatin reversed the expression of a large number of mRNAs and long non-coding RNAs in human gastric cancer drug-resistant cells. The molecular mechanism of inhibiting the proliferation of human gastric cancer drug-resistant cells may be related to the upregulation of long non-coding RNAs and mRNAs contributed in CD95, mTOR, and TNF receptor signaling pathway.*

Keywords: *Cisplatin, gastric cancer, hyperthermia, long non-coding RNA, proliferation*

INTRODUCTION

According to the global cancer statistics released in 2018, gastric cancer (GC) is the fifth most malignant tumor in the world and the third leading cause of cancer-related death.[1-3] However, patients with GC often miss a proper treatment opportunity, leading to the advanced development of the disease.[4,5] Patients with GC often show that getting a complete remission is a difficult process, and they are prone to metastasis.[6] At present, chemotherapy, molecular-targeted therapy, and immunotherapy have been used as the primary treatment options to treat patients with advanced GC. Recently,

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human biology and genetics studies of tumor cells showed that selective therapies are the promising treatment options; however, the mechanism of how genetics could be used to treat GC is yet to be understood.

The common treatment for patients with advanced GC is chemotherapy. It has been suggested that platin-containing regimens should be the first-line treatment for GC,[7-11] and it could have a synergistic effect in combination with a variety of chemotherapeutics.[12] In addition to chemotherapy, high temperature has direct cytotoxicity and can improve the penetration of chemotherapy into tissues[13]; it could be used alongside chemotherapy, due to synergistic effects.[14,15] At the same time, the regulatory effect of hyperthermia (HT) in the resistance mechanism of GC cells to cisplatin has been demonstrated.[16] It can be seen that HT-assisted GC chemotherapy has the function of enhancing chemotherapy sensitivity and alleviating drug resistance, which coincides with the purpose of achieving synergistic effects in clinical GC patients.[17-19]

It has been well documented that RNA molecules carry genes and regulatory information and can reflect the real-time state of cells.[20] In this regard, it has been found that non-coding RNA miRNA-218 was upregulated 8-fold higher in patients with GC after undergoing hyperthermic perfusion therapy and enhanced the sensitivity of GC cells SGC-7901 to cisplatin chemotherapy in vitro.[21,22] For now, long non-coding RNA (lncRNA) is increasingly used as a promising tumor diagnostic marker due to its complex functional mechanism.

At present, there are few reports on the molecular mechanism supporting HT combined with chemotherapy to inhibit the proliferation of human GC resistant cells SGC-7901/DDP.[23-26] In this regard, the current study aims to evaluate the effects of HT in combination with cisplatin (DDP) on the inhibition of human GC drug-resistant cell SGC-7901/DDP in vitro and explore its possible molecular mechanism. For reaching this goal, we used high-throughput ChIP-seq detection technology to explore the synergistic inhibition of SGC-7901/DDP by HT and cisplatin. The results of the current study could explore the molecular mechanism of cell proliferation and provide a reference for the diagnosis and clinical treatment for GC patients.

Main Points

- *Hyperthermia combined with cisplatin increased the rate of early apoptosis of SGC-7901/DDP cells.*
- *Hyperthermia combined with cisplatin reversed the expression of a large number of mRNAs and long non-coding RNAs in human gastric cancer drug-resistant cells.*
- *The molecular mechanism of inhibiting proliferation of human gastric cancer drug-resistant cells may be related to upregulation of TCONS_00018082 and ENST00000412526.1 and downregulation of TCONS_00015171 and ENST00000584911.1.*

MATERIALS AND METHODS

Experimental Design

As mentioned in Figure 1, we evaluated the possible molecular mechanism of thermo-chemotherapy in drug-resistant human GC cell SGC-7901/DDP in vitro. We divided the cells into 4 groups: control group, cisplatin group, HT group, and HT combined with cisplatin group. High-throughput ChIP-seq and real-time fluorescence quantitative polymerase chain reaction (RT-PCR) were used for evaluating the apoptotic rate of SGC-7901/DDP cells, lncRNAs expression and verification, respectively.

Cells and Cell Culture Conditions

Human GC drug-resistant cell line SGC-7901/DDP was provided by Department of Gastroenterology, Shangrao People's Hospital, Shuinan Jiedao Jiangxi Province, China. We transferred the cells to a centrifuge tube containing 10% fetal bovine serum and RPMI-1640 complete medium, and adjust the centrifuge speed to 800 rpm, and centrifuged for 5 minutes. We discarded the supernatant and added the complete medium; then the cells were transferred to the culture flask and incubated at 37°C with 5% CO_2 incubator.

We divided the human GC drug-resistant cells SGC-7901/DDP into the following 4 groups:

1. Control group: Routine culture in a 37°C incubator without other intervention.
2. Cisplatin group: Cisplatin with complete medium with the concentration of cisplatin at 1 μg/mL, 2 μg/mL, and 3 μg/mL.
3. HT group: Adjust the temperature to 42°C, 44°C, 46°C, 48°C, and 50°C put it in a sealed cell culture dish and heat it, sterilize and put it in the incubator after an hour.
4. HT combined with cisplatin group: Cisplatin for 8 h → water bath heating for 1 hour; the incubation time started from the end of the heating and exceeded 12 hours, and it heated once every 12 hours.

MTT Assay

For evaluating the cell viability test to detect cell proliferation, we used MTT assay by adding 20 μL of 0.5%

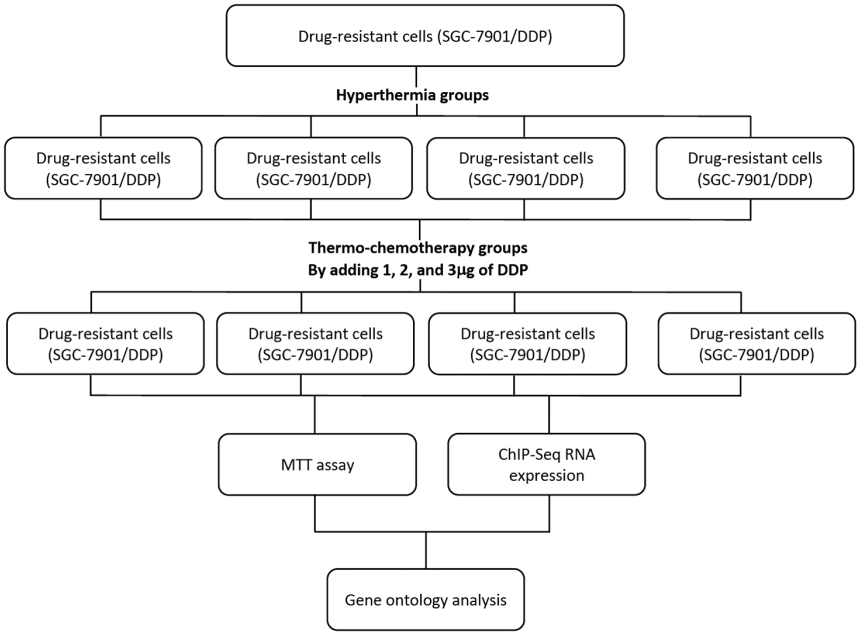

Figure 1. Experimental design flow chart.

MTT to each well and incubating for 4 hours in an incubator, based on the manufacturer's instruction; then we measured the absorbance OD value. To assess the MTT assay, briefly, cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin and incubated in 5% CO_2 incubator at 37°C. The medium was renewed every 2 days until the monolayer cultures reached between 70% and 80% confluence. Then, the cells were washed with phosphate-buffered saline (PBS), followed by incubation with trypsin for 5 minutes. The trypsin was deactivated by the addition of DMEM supplemented with fetal bovine serum, and the cell suspension was centrifuged at 3000 rpm for 5 min at 20°C. The resulting supernatant was removed, and medium was added and mixed for resuspension of cells, which were used for MTT assay.

Isobaric Diagram Method to Detect Synergy

In order to determine whether the combined effect of HT and cisplatin has a synergistic effect, an isobaric map analysis was performed by using MTT assay. According to the analysis, the interaction between different HT temperature and cisplatin concentration is measured according to the combination index (CI): when CI < 1, they have a synergistic effect, when CI = 1, they have an additive effect, and when CI > 1, they have an antagonistic effect.

High-Throughput ChIP-Seq for Detecting Long Non-coding RNA Expression

In this step, we extract RNA as follows: first, SGC-7901/DDP cells were washed twice with cooled PBS solution, then 1 mL of Trizol was added, and the mixture was stored in Expand to Eppendorf tube. Then, we added 200 μL of chloroform and centrifuged at 12 000 rpm at 4°C for 15 minutes and the supernatant was discarded, and this was again centrifuged at 4°C, at 5000 rpm for 5 min, and Diethyl pyrocarbonate was added to dissolve RNA and stored at −80°C. Now the samples were ready for synthesizing of cDNA from RNA. For synthesizing of cDNA, a solution of reverse transcriptase buffer was added. In this regard, we started the PCR and adjust the reaction conditions to: 16°C → 1 hour, 65°C → 10 minutes, and 4°C → 5 minutes. After the PCR reaction, samples were stored at −20°C.

Gene Ontology Analysis

Gene ontology (GO) analysis mainly annotates the functions of differential genes and can find RNA molecules related to changes in the functions of the specific genes, which is helpful for later experimental results and research. Gene ontology analysis includes 3 aspects: molecular function, cell composition, and biological process. Each aspect contains different gene function items, and the degree of enrichment of each item is different. This topic uses FunRich 3.1.3 software to perform GO analysis on the selected differentially expressed (DE)-mRNA.

The software analyzes the number of genes, gene ratios, enrichment multiples, mapped gene names, and *P* values in each entry. $P < .05$ is the criterion for the significant enrichment of the entries of DE genes. According to the *P* value, the degree of enrichment of each item can be clarified, which is convenient for finding highly enriched gene items and determining the most significant gene function.

Statistical Analysis

GraphPad Prism 8.0 software is used for analysis and drawing, and The Statistical Package for Social Sciences (SPSS) version 24.0 software (IBM Corp.; Armonk, NY, USA) is used for statistical analysis of MTT data and the relative expression of lncRNA. When the data obey a normal distribution, it is represented by the mean ± standard deviation (±SD). *P* value less than .05 was considered as significant.

RESULTS

Evaluation of Cell Proliferation Under Different Hyperthermia Time and Temperature MTT Assay

The results of the MTT experiment showed that the cell proliferation inhibition rate increased with HT and the extension of the time, as shown in Figure 2. The inhibition rate reached at least 40% after 48 hours of HT at 44°C. When the temperature of HT reached 46°C, the cell proliferation inhibition rates after 12 hours, 24 hours, and 48 hours are 21.75%, 46.17%, and 53.75%, respectively. Based on the treatment of HT combined with cisplatin, the cells grew too densely after 48 hours of intervention, which could result in inhibitory factors rather than the intervention factors. In this regard, 24 hours was selected as the culture time for the combination of HT and cisplatin.

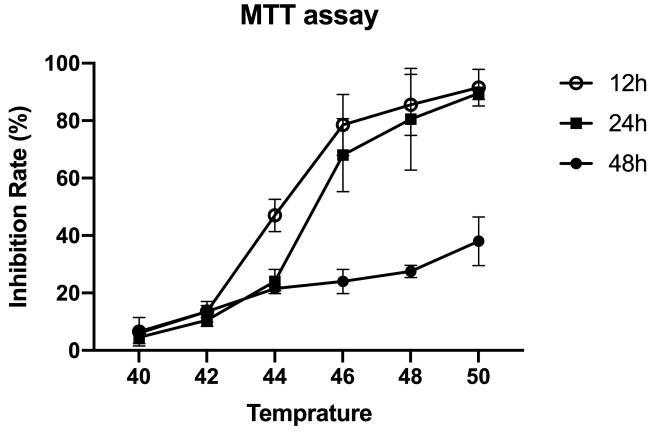

Figure 2. Results of MTT assay. Cell proliferation inhibition rate with hyperthermia temperature and hyperthermia time curve.

Analyzing Synergistic Effect of Hyperthermia Combined with Cisplatin

According to the CI value results, combination of 42°C and 44°C and 1 μg/mL, 2 μg/mL, and 3 μg/mL of cisplatin, respectively, could not inhibit the cell proliferation (Figure 3); similarly, combination of 1 μg/mL cisplatin at 46°C did not inhibit the cell proliferation. However, HT at 46°C in combination with 2 μg/mL and 3 μg/mL of cisplatin inhibited the cell proliferation. The inhibition was significantly stronger with 3 μg/mL of cisplatin at 46°C in comparison to 2 μg/mL of cisplatin at 46°C. In addition, we observed that the combination of HT at 46°C and 3 μg/mL of cisplatin could inhibit cell proliferation rate higher than 60%.

Hyperthermia Combined with Cisplatin Affects the Early Apoptosis of SGC-7901/DDP Cells

Based on the above culture conditions, the apoptosis of SGC-7901/DDP cells after different interventions is shown in Figure 4. The results showed that cisplatin and HT induced 4.16% and 11.52% of early apoptosis of SGC-7901/DDP cells. In comparison to the HT group, the early apoptosis rate of cells in the HT combined with cisplatin group increased nearly 2.1 times (24.31%, Figure 4). It can be seen that cisplatin has no significant impact

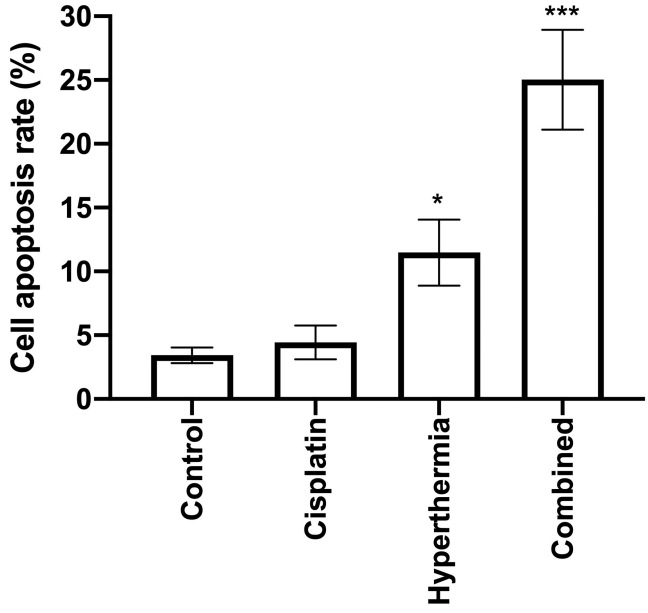

Figure 3. Early cell apoptosis rate in the control group, cisplatin group, hyperthermia group, and hyperthermia combined with cisplatin group. **P* < .05 compared with the cisplatin group and ****P* < .001 compared with the hyperthermia group.

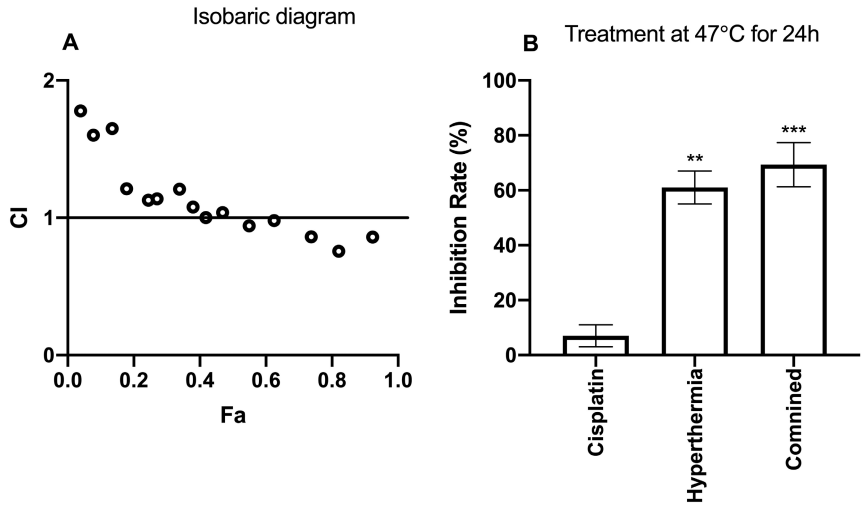

Figure 4. The synergic effect of hyperthermia combined with cisplatin on the proliferation inhibition rate of human gastric cancer resistant cell SGC-7901/DDP. (A) Isobaric diagram of the combined effect of different hyperthermia temperatures and different cisplatin concentrations; (B) Cell proliferation inhibition rate of each group after treatment at 47°C for 24 hours (**$P < .01$: compared with cisplatin group, ***$P < .001$: compared with hyperthermia group comparison).

on the early apoptosis of cells, but HT combined with cisplatin could increase the early apoptosis rate of cells. Hyperthermia enhanced the sensitivity of cisplatin to interfere with SGC-7901/DDP cells.

RNA Expression of SGC-7901/DDP Cells in Combination of Hyperthermia and Cisplatin

Cluster analysis of the data obtained from high-throughput ChIP-seq detection revealed that the expression of lncRNA and mRNA in HT combined with cisplatin group and the control group was significantly different from that observed in cisplatin group and the control group (Figure 5). The microarray results showed that the upregulated lncRNA and mRNA in the control group were downregulated after combination of the HT and cisplatin. In comparison to the control group, the HT combined with cisplatin group had 8730 DE-lncRNAs and 7194 of DE-mRNAs. Regarding DE-lncRNAs, there were 5093 upregulated and 6961 downregulated genes, while DE-mRNAs are upregulated in 1983 cases and down-regulated in 4092. These results were reversed in a large number of genes when we treated SGC-7901/DDP cells with 2 μg/mL of cisplatin combined with 46°C HT. The top 10 upregulated and downregulated mRNAs and lncRNAs are listed in Table 1.

Gene Ontology Analysis

Gene ontology analysis revealed that DE-mRNAs are associated with many specific biological pathways (e.g., immune system, TNF receptor signaling pathway, and mTOR signaling pathway) and cellular components (e.g., nuclear reactions). The upregulation of DE-mRNAs was highly related to immune system response and CD95 signaling pathway in nucleus, and downregulation of

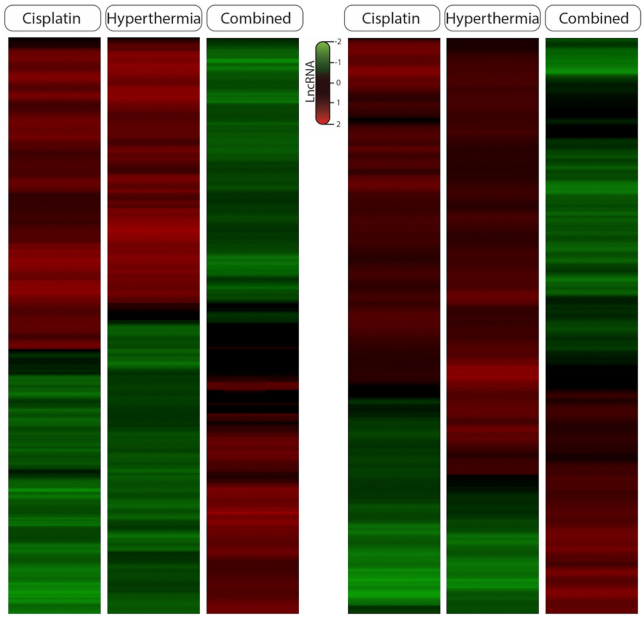

Figure 5. The differential expression of long non-coding RNAs (left) and mRNAs (right) in the hyperthermia combined with the cisplatin group compared to the control group. (A) Heat map of differentially expressed (DE)-lncRNAs; (B) Heat map of DE-mRNAs (red means downregulation and green means upregulation).

Table 1. Basic Information of the Top 5 Dysregulated mRNAs and lncRNAs

lncRNAs		mRNAs		
Probe	Gene ID	Gene Name	Entrez ID	Final Regulation
p40905_v4	XR_241959.1	PARP1	142	Upregulate
p23776	TCONS_00013003	CFLAR	8837	Upregulate
p22196	TCONS_00006308	HSPA6	3310	Upregulate
p29247	ENST00000424748.1	C10orf10	11067	Upregulate
p23751	TCONS_00013378	SCGB1C1	147199	Upregulate
p14787	ENST00000598092.1	VWA5A	4013	Downregulate
p12964	ENST00000551672.1	CRTAC1	55118	Downregulate
p27518	ENST00000444665.1	RBP1	5947	Downregulate
p25173	ENST00000435810.1	IGIP	492311	Downregulate
p10147	ENST00000455598.1	KDM4C	23081	Downregulate

lncRNAs, long non-coding RNAs.

DE-mRNAs was highly related to mTOR and TNF receptor signaling pathway in cytoplasm (Figure 6).

DISCUSSION

In this study, double staining showed that HT combined with cisplatin increased the rate of early apoptosis of SGC-7901/DDP cells. High-throughput ChIP-seq of lncRNA showed a significantly larger amount of lncRNAs and mRNAs in the cells treated with HT combined with cisplatin group in comparison to the control group, and RT-PCR test results confirmed that lncRNA TCONS_00018082 and ENST00000412526.1 were significantly upregulated, while the relative expression levels of lncRNA TCONS_00015171 and ENST00000584911.1 were significantly downregulated.

It has been shown that HT can relieve tissue hypoxia and can be used simultaneously with radiotherapy or anticancer drugs to enhance its cytotoxic effect on tumors.[27] As a traditional anti-tumor method, HT can inhibit tumor cell invasion and metastasis and promote cell apoptosis.[28] Hyperthermia combined with chemotherapy can significantly improve the survival rate and tumor growth control of patients.[27,28] However, a number of studies on hyperthermia have shown that radiation-induced HT can cause breast cancer, colon cancer, bladder cancer, nasopharyngeal cancer, and so on, resistance to chemotherapy.[29-32] In this regard, Xie Dan et al[33] found that HT can promote the key mediator of cisplatin resistance-nucleotide excision repair cross complementation group 1, which is significantly at downregulated mRNA and induced apoptosis in ovarian cancer cells.[33] In this regard, this study designed temperature gradients of 41°C, 44°C, 47°C, and 50°C to study the interaction between cisplatin and different HT temperatures and evaluate its effects on the proliferation and apoptosis of SGC-7901/DDP cells. We found that 47°C HT combined with 2 μg/mL cisplatin has a better synergistic inhibitory effect.

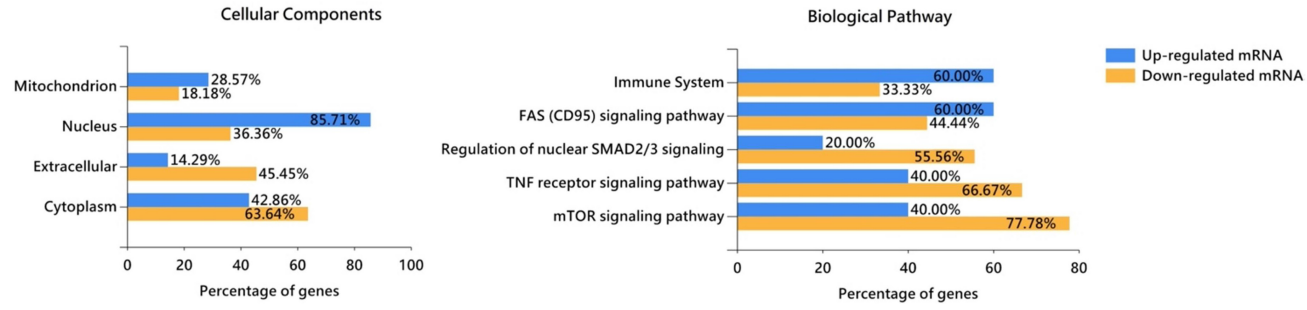

Figure 6. Differentially expressed (DE) mRNA gene ontology analysis of hyperthermia combined with cisplatin group compared to control group. (A) Upregulation of DE-mRNA enrichment entry histogram; (B) Downregulation of DE-mRNA enrichment entry histogram.

It has been well documented that inhibiting the proliferation of cancer cells and inducing normal apoptosis of cancer cells is essential for anti-tumor therapy.[34] In this study, MTT assay and flow cytometry were used to detect the effects of HT combined with cisplatin group, cisplatin group, and HT group on cell proliferation and early apoptosis. The results showed that combination group and HT group had more obvious inhibitory effects on cell proliferation in comparison to cisplatin group with regard to cell proliferation ability. The results of cell apoptosis analysis found that the number of cells undergoing early apoptosis in the combination group was significantly higher than that of the 2 separate intervention groups. Annexin V-Fluorescein isothiocyanate/Propidium iodide (FITC/PI) used in this study is a proper reagent for studying tumor apoptosis (no cells undergo middle and late stages of apoptosis or necrosis).[34] Through these 2 experiments, we conclude that the combined treatment of 2 μg/mL cisplatin and 47°C HT significantly promotes early cell apoptosis and has a synergistic effect on cell SGC-7901/DDP to inhibit cell proliferation.

When the regulatory RNA is abnormally expressed, the function of the RNA itself and its post-transcriptional expression will be abnormal, and the mRNA level of the encoding oncogene will also fluctuate, eventually changing the function of tumor suppressor genes and proto-oncogenes and causing tumors. The abnormal regulation of RNA in tumor cells will lead to specific changes in the RNA expression profile and related genes or proteins. This study uses high-throughput ChIP-seq expression profiles to detect RNA expression profiles in SGC-7901/DDP cells after intervention in the control group, cisplatin group, and combined group. The experimental results showed that compared with the control group, there was almost no difference in the RNA expression profile of the cisplatin group, while the combination of HT and cisplatin caused a completely opposite change in the cellular RNA expression profile, and many different RNAs were oppositely regulated. Gene ontology analysis is often used to explain gene function from 3 aspects: biological process, cell composition, and molecular function.[35] Upregulation of mRNA is mainly involved in the immune response of SGC-7901/DDP cells, and heat shock protein (HSP) activity, antigen binding, receptor activity, and Major histocompatibility complex (MHC) I/II activity is related. Among them, HSP peptide complexes are often formed by the combination of HSP and antigen peptides and participate in the immune process. The immune response of HSP peptide complexes in tumor cells is tumor-specific in inducing the immune effect of cytotoxic T lymphocytes and does not rely on antigen extraction.[36,37] These results indicate that HT combined with cisplatin may cause changes in the composition of the extracellular matrix and affect the activity of HSP, MHC I/II, and other substances in the process of SGC-7901/DDP cellular immune response. Downregulation of mRNA is mainly involved in the regulation of nucleic acid metabolism, endocytosis, gene expression, cell cycle and apoptosis, biological process, molecular function and DNA binding, ubiquitin-specific protease (USP) activity, and phosphodiester hydrolase activity.

Among them, USP activity is closely related to the occurrence and development of tumors. Ubiquitin-specific protease is a deubiquitinating enzyme, and among more than 50 members, many are closely related to the occurrence and development of malignant tumors[38]; therefore, it is used as an effective way to find new targeted treatments for malignant tumors. It has been found that the USP family has the function of regulating the sensitivity of cancer cells to chemotherapeutics. For example, USP14 enhances the resistance of ovarian cancer cells to cisplatin by downregulating the BCL6 protein,[39] USP7 enhances the drug resistance of liver cancer cells,[40] USP4 enhances the drug resistance of lung cancer cells,[41] and so on. The regulation of USP activity is expected to become an effective therapeutic target for alleviating the tumor drug resistance.

Pathway analysis showed that upregulation of differential mRNA is mainly involved in G-protein-coupled receptors (GPCRs)-ligand signaling pathway, rhodopsin-like receptor signaling pathway, polypeptide receptor-ligand signaling pathway, and chemokine receptor-chemokine signaling pathway. GPCRs that only exist in eukaryotes include both protein-like macromolecular substances and polypeptide-like small molecules, which are widely involved in the transduction of signal molecules inside and outside the cell. GPCRs bind to chemokines and other signal molecules to activate biological signal transduction pathways in cells to change the state of cells. Rhodopsin, as a kind of GPCRs, also participates in the above-mentioned signal transduction. For example, Ding et al[42] found that the combination of CXCR-3 in the rhodopsin superfamily with the chemokine receptor CXCL9 caused significant changes in the phosphorylation level of ERK1/2 in the MAPK signaling pathway, regulating tumor cell invasion and metastasis.[42] Studies have shown that chemokines and their related GPCRs work together to control the positioning of immune cells in time and space. For example, CXCL7 plays a central role

in the coordination between mature antigen-presenting cells and naive T cells. The process is the key to initiating the cellular immune response.[43] In addition, downregulation of differential mRNA mainly regulates the cell cycle of tumor cells (M-phase and M-M/G1 phase). Also, BARD1 signaling pathway and TRAIL signaling pathway are significantly enriched. BARD1 is a key molecule that maintains genome stability. The ligase activity of BRCA1 and BARD1 is a key regulator of DNA repair, but its role in growth signal transduction is unclear.[44] Studies have shown that overexpression of BARD1 in liver cancer and breast cancer can promote tumor cell proliferation, which is related to the poor prognosis of early breast cancer patients receiving radiotherapy.[21,44]

There are different types of lncRNAs in tumor cells, and they are different in structure and function. This study classified the DE lncRNAs in the high-throughput microarray expression profile. Non-coding RNAs with base pairs greater than 200 nt are lncRNAs, and non-coding RNAs over 10 kb are ultra-long lncRNAs. Among them, lncRNAs account for more than 90%. We found that many lncRNAs are related to the regulation of radiotherapy and chemotherapy tolerance. For example, ENST00000592689.1 is downregulated after HT combined with cisplatin intervention, but its upregulation is closely related to the occurrence and development of a variety of tumors. In osteosarcoma, it regulates the miR-134-5p/MBTD1 signal axis on osteosarcoma cell apoptosis, produces inhibition, and reduces the sensitivity of osteosarcoma cells to cisplatin chemotherapy [84]. The downregulated lncRNA ENST00000585415.1 may inhibit the growth or apoptosis of tumor cells by inhibiting the expression of Basic metabolic panel (BMP) and reducing the radiation tolerance of nasopharyngeal carcinoma.[45] However, ENST00000466856.1, as a tumor suppressor factor, is upregulated after combined treatment. It can inhibit the proliferation of endometrial cancer cells by targeting silencing miR-23b and enhancing the interventional efficacy of cisplatin.[46] Overexpression of lncRNA ENST00000412526.1 in osteosarcoma cells can reverse cisplatin resistance and enhance apoptosis by upregulating IFIT2, but its ability to regulate cisplatin resistance in ovarian cancer is the opposite [87,88]. These lncRNAs have not been studied in GC, but ENST00000584911.1 is related to the malignant state and prognosis of GC. It is an oncogenic lncRNA that regulates the migration and invasion of GC cells. After downregulation, it inhibits tumor cell proliferation and enhances tumors.[47] The role of cell chemosensitivity. The above findings indicate that the combination of HT and cisplatin regulates the expression of these lncRNAs in the direction of reversing the drug-resistant phenotype of tumor cells and inhibiting cell proliferation.

CONCLUSION

Hyperthermia at 46°C in combination with 2 μg/mL cisplatin for 24 hours has a synergistic inhibitory effect on the cell proliferation of resistant GC cells SGC-7901/DDP and promotes early cell apoptosis. Hyperthermia combined with cisplatin caused differential expression of a large number of mRNAs and lncRNAs in GC drug-resistant cells. DE mRNA may be involved in multiple processes such as immune response, apoptosis, and drug resistance regulation. The molecular mechanism of inhibiting the proliferation of human GC drug-resistant cells may be related to upregulation of lncRNAs and mRNAs contributed in CD95, mTOR, and TNF receptor signaling pathways.

Ethics Committee Approval: The study was approved by the medical ethics committee of Nanjing Medical University (No: R2783).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Consept – X.F.; Design – F.A.Z.; Supervision – X.F., M.A.R.; Resources – F.A.Z.; Materials – F.A.Z.; Data Collection and/or Processing – F.A.Z., H.L.; Analysis and/or Interpretation – H.L., Y.W.; Literature Search – Y.W.; Writing Manuscript – F.A.Z., J.Y.; Critical Review – F.A.Z., R.A.S.

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