

Basic Study

ECRG2 enhances the anti-cancer effects of cisplatin in cisplatin-resistant esophageal cancer cells *via* upregulation of *p53* and downregulation of *PCNA*

Xin-Fang Hou, Lin-Ping Xu, Hai-Yan Song, Shuai Li, Chen Wu, Ju-Feng Wang

Xin-Fang Hou, Shuai Li, Chen Wu, Ju-Feng Wang, Department of Medical Oncology, Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, Zhengzhou 450008, Henan Province, China

Lin-Ping Xu, Department of Science and Research, Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, Zhengzhou 450008, Henan Province, China

Hai-Yan Song, Key Laboratory for Medical Tissue Regeneration of Henan Province, College of Basic Medicine, Xinxiang Medical University, Xinxiang 453003, Henan Province, China

Supported by the Public Welfare Project Foundation of Henan Province, No. 20130010.

Institutional review board statement: The study was reviewed and approved by the Henan Cancer Hospital Institutional Review Board.

Conflict-of-interest statement: To the best of our knowledge, no conflict of interest exists.

Data sharing statement: There are no additional data available in relation to this manuscript.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Dr. Ju-Feng Wang, Professor, Department of Medical Oncology, Affiliated Cancer Hospital of Zhengzhou University, Henan Cancer Hospital, 127 Dongming Road, Zhengzhou 450008, Henan Province, China. 13783583966@163.com

Telephone: +86-371-65587598
Fax: +86-371-65587598

Received: November 30, 2016
Peer-review started: December 2, 2016
First decision: December 19, 2016
Revised: January 6, 2017
Accepted: January 18, 2017
Article in press: January 18, 2017
Published online: March 14, 2017

Abstract**AIM**

To explore the anti-tumor effects of esophageal cancer-related gene 2 (ECRG2) in combination with cisplatin (DDP) in DDP-resistant esophageal cancer cells (EC9706/DDP).

METHODS

A drug-resistant cell model was established, with EC9706/DDP cells being treated with ECRG2 and/or DDP. Cell viability was examined by MTT assay. The rate of cell apoptosis was determined by flow cytometry. The mRNA expression levels of proliferating cell nuclear antigen (PCNA), metallothionein (MT), and p53 were determined by RT-PCR and PCNA, while MT and p53 protein expression levels were determined by western blotting.

RESULTS

The anti-proliferative effect of ECRG2 in combination with DDP was superior when compared to ECRG2 or DDP alone. The inhibition rate for the combination reached its peak (51.33%) at 96 h. The early apoptotic rates of the control, ECRG2 alone, DDP alone, and ECRG2 plus DDP groups were 5.71% ± 0.27%, 12.68% ± 0.61%, 14.15% ± 0.87%, and 27.96% ±

0.36%, respectively. Although all treatment groups were significantly different from the control group ($P < 0.05$), the combination treatment of ECRG2 plus DDP performed significantly better when compared to either ECRG2 or DDP alone ($P < 0.05$). The combination of ECRG2 and DDP significantly upregulated p53 mRNA and protein levels and downregulated PCNA mRNA and protein levels compared to ECRG2 or DDP alone ($P < 0.05$). However, no changes were seen in the expression of MT mRNA or protein.

CONCLUSION

ECRG2 in combination with DDP can inhibit viability and induce apoptosis in esophageal cancer DDP-resistant cells, possibly *via* upregulation of p53 expression and downregulation of PCNA expression. These findings suggest that the combination of ECRG2 and DDP may be a promising strategy for the clinical treatment of esophageal cancers that are resistant to DDP.

Key words: Esophageal cancer related-gene 2; Cisplatin; Resistance; p53; Proliferating cell nuclear antigen

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Cisplatin (DDP) is one of the most effective agents in treating esophageal cancer, but drug resistance poses a major impediment. Combination therapy has become an important method for overcoming drug resistance. This study showed that esophageal cancer-related gene 2 (ECRG2) in combination with DDP can inhibit viability and induce apoptosis in DDP-resistant esophageal cancer cells, possibly *via* upregulation of p53 expression and downregulation of proliferating cell nuclear antigen expression. These findings suggest that the combination of ECRG2 and DDP may be a promising strategy for the clinical treatment of esophageal cancers that are resistant to DDP.

Hou XF, Xu LP, Song HY, Li S, Wu C, Wang JF. ECRG2 enhances the anti-cancer effects of cisplatin in cisplatin-resistant esophageal cancer cells *via* upregulation of p53 and downregulation of PCNA. *World J Gastroenterol* 2017; 23(10): 1796-1803 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v23/i10/1796.htm> DOI: <http://dx.doi.org/10.3748/wjg.v23.i10.1796>

INTRODUCTION

Esophageal cancer is a severe global health problem with an incidence that ranks eighth among all of the malignant cancers^[1]. In spite of improvements in early-stage detection, most tumors are already at an advanced stage upon initial diagnosis^[2]. Cisplatin (DDP)-based doublets have been the most commonly used treatment regimen for most patients with locally advanced or

metastatic esophageal cancer^[3-6]. Unfortunately, DDP resistance and dose-limiting side effects have been a challenge to any further improvement of this treatment's efficacy. These obstacles call for a novel combination of therapeutic approaches in order to achieve high therapeutic efficacy at a lower drug dose^[7].

Esophageal cancer related gene 2 (ECRG2) was first separated and identified, using the mRNA technique of differential display, in normal esophageal tissues and esophageal cancer tissues in 1998^[8]. It has been reported that ECRG2 inhibits tumor cell growth and promotes cell apoptosis *in vivo* and *in vitro*^[9,10]. In our previous study, we found that ECRG2 combined with DDP had an enhanced inhibitory effect on EC9706 cell proliferation and an increased inductive effect on EC9706 cell apoptosis when compared to ECRG2 alone^[11]. This suggests that ECRG2 may be involved in the drug resistance of esophageal cancer. There are currently no known studies in the literature examining the effects of ECRG2 on the drug resistance of esophageal cancer. In the present study, we established a drug-resistant cell model to explore the effects of ECRG2 on drug resistant cells.

MATERIALS AND METHODS

Cell culture and reagents

PCNA, p53, and MT primers and ECRG2 protein were manufactured by Sangon Biological Engineering Co., Ltd. (Shanghai, China). DDP was bought from Qilu Pharmaceutical Co., Ltd. (Jinan, China). RPMI 1640 medium and fetal bovine serum (FBS) were purchased from HyClone (Logan, UT, United States). 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), TRIzol, and annexin V-FITC apoptosis detection kits were purchased from Sigma Co. (St. Louis, MO, United States). The reverse transcription and amplification kits were purchased from Promega Co. (Madison, WI, United States). The rabbit anti-human PCNA and p53 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, United States). The rabbit anti-human MT and goat anti-rabbit IgG antibodies were purchased from Abcam (Cambridge, United Kingdom). The human esophageal cancer cell line EC9706 was provided by the tumor cell library of the Academy of Medical Sciences in China.

Establishment of a drug-resistant cell line

We shocked EC9706 cells in the logarithmic phase with a final concentration of 2 mg/L of DDP. We discarded the culture solution after 48 h, washing 3 times with PBS. The cells were cultured in a fresh nutrient solution and washed every 2 d to remove dead cells. When the cells reached stable growth, we increased the DDP concentration, and repeated until the cells maintained growth in a medium with a concentration of 0.5 mg/L of DDP. The cells were cultured in a RPMI-1640 medium containing 10% fetal bovine serum, 100 U/m penicillin,

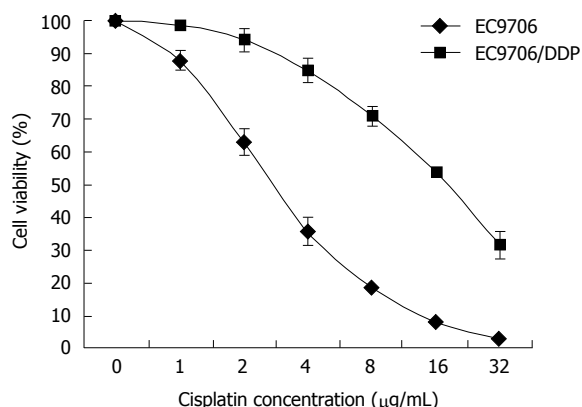


Figure 1 Survival rate of EC9706 and EC9706/cisplatin (DDP) cells treated with various concentrations of DDP. A dose-dependent association between the survival rate of EC9706 and EC9706/DDP cells and the concentration of DDP can be observed. The survival of EC9706 cells was significantly lower compared to EC9706/DDP cells following treatment with various concentrations of DDP. The IC50 of DDP in EC9706 cells is 3.34 ± 0.27 µg/mL, while the IC50 of DDP in EC9706/DDP cells is 16.83 ± 1.46 µg/mL.

100 U/mL streptomycin, and 0.5 mL/L DDP in a humidified incubator at 37 °C with 5% CO₂.

Cell viability

Drug sensitivity was analyzed by MTT assay. The cells were plated at 1×10^3 cells/mL in 96-well plates in a volume of 100 µL and incubated for 24 h. The media were discarded and cells were treated with various concentrations of DDP or ECRG2 for 72 h. A total of 20 µL of 5 g/L MTT solution was added to each well, with cells then being incubated for 4 h at 37 °C. The medium was discarded and 150 µL of dimethyl sulfoxide was added to each well, which was then shaken to completely dissolve the blue-purple precipitate from the MTT. A microplate reader was used to test the absorbance (OD) of each well at 490 nm and average values were obtained. Cell viability was calculated using the formula: $(\text{OD treated well} - \text{OD blank}) / (\text{OD control well} - \text{OD blank}) \times 100$.

Cell apoptosis assay using annexin-V FITC/PI

The cells were seeded in a 6-well plate at a density of 4×10^5 cells per well. After overnight attachment, cells were treated with ECRG2 and/or DDP for 24 h. The cells were then harvested by trypsinization, washed with cold PBS, centrifuged at 1×10^3 rpm for 5 min, and resuspended in 1 × binding buffer. 500 µL of each sample solution was then added to 5 µL of FITC-conjugated annexin-V and 10 µL of PI, followed by incubation for 15 min in the dark at room temperature. The samples were then measured by flow cytometry.

RNA isolation and RT-PCR

Each group of cells was collected after 24 h of drug treatment. The total RNA was extracted from each group of cells using TRIzol reagent according to the manufacturer's protocol. The cDNA was made *via* the protocol for the Reverse Transcription System for RT-

PCR. The primers for PCNA were 5'-ACCGCTGCGACC GCAATTTG-3'(forward) and 5'-ACGTGCAAATTCACC AGAAGGCATC-3'(reverse). The primers for MT were 5'-CGGATCCAGACTCAAACAGGCTTTTAT-3' (forward) and 5'-CGGATCCCCGGAATGGACCCCAACTGCT-3' (reverse). The primers for p53 were 5'-ATTGCGTGTG GAGTATTGG-3' (forward) and 5'-GCTGTTCCGTC CAGT AGATTA-3' (reverse). The primers for GAPDH were 5'-TCATGGGTGTGAACCATGAGAA-3' (forward) and 5'-GGCATGGACTGTGGTCATGAG-3' (reverse). PCR amplification was performed using a kit under the following conditions: 95 °C for 5 min, followed by 30 cycles at 95 °C for 40 s, 55 °C for 40 s, and 72 °C for 1 min. The expression level of mRNA encoding the p53, PCNA, and MT genes were evaluated and normalized relative to that of GAPDH.

Western blot

The cells were collected from each group and lysed in 200 µL of 1% Tween 20 in PBS by five cycles of freezing and thawing. The insoluble proteins were removed by centrifugation. After quantification of the total protein, the protein was separated on 8% polyacrylamide gel (PAGE), and transferred to a PVDF membrane (Millipore). The membranes were then blocked with 5% non-fat dry milk in PBS-Tween 20 for 1 h at room temperature. After incubation with polyclonal rabbit anti-human MT, PCNA, or p53 antibody overnight at 4 °C at a 1:500 dilution, the membranes were incubated with a secondary HRP-conjugated anti-rabbit antibody at a 1:1000 dilution and then washed again three times with PBST buffer. The transferred proteins were visualized using an ECL detection kit according to the manufacturer's protocol.

Statistical analysis

The SPSS 13.0 software system (SPSS, Inc., Chicago, IL, United States) was used for statistical evaluation. The data are expressed as the mean ± SD for at least 3 independent experiments. Statistical significance was evaluated by analysis of variance (ANOVA) with Tukey's post hoc test. A *P* value of < 0.05 was considered to indicate a statistically significant result.

RESULTS

Establishment of drug-resistant, EC9706/DDP cells

The EC9706/DDP cell line was established after several months of intermittent exposure by the parental cells to a gradually increasing concentration of drugs. After the EC9706 and EC9706/DDP cells were treated with DDP at various concentrations (1, 2, 4, 8, 16, 32 µg/mL) for 48 h, the cell viability rate was examined by MTT assay. As shown in Figure 1, the IC50 for 48 h of DDP in the EC9706/DDP cells was 16.83 ± 1.46 µg/mL and the IC50 for 48 h of DDP in the parental EC9706 cells was 3.34 ± 0.27 µg/mL. Therefore, the EC9706/DDP cells were five times more resistant to DDP compared to the parental cells.

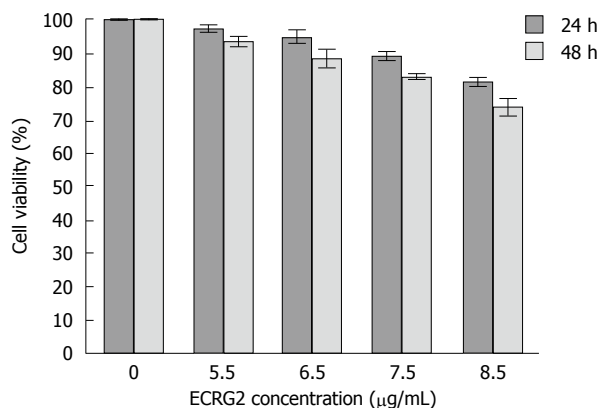


Figure 2 Survival rate at various concentrations of esophageal cancer-related gene 2. A dose- and time- dependent association between the survival rate of the EC9706/cisplatin (DDP) cells and the concentration of ECRG2. A longer incubation time with ECRG2 significantly decreased the survival rate of EC9706/DDP cells. The same was found for treatments with a higher concentration of ECRG2.

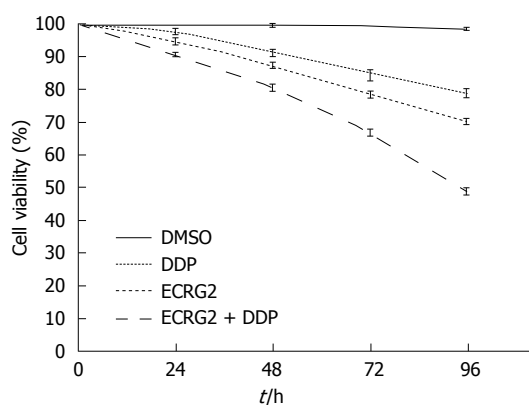


Figure 3 Effects of esophageal cancer-related gene 2 combined with cisplatin (DDP) on cell viability in EC9706/DDP cells. Cells were treated with dimethyl sulfoxide, ECRG2 (6.5 µg/mL) alone, DDP (3 µg/mL) alone, or a combination of ECRG2 (6.5 µg/mL) and DDP (3 µg/mL) for 24 h, 48 h, 72 h, and 96 h. Cell viability was detected via MTT assay.

Effects of ECRG2 on the EC9706/DDP cells

To evaluate the effects of ECRG2 on the growth and viability of the cells, the EC9706/DDP cells were treated with various concentrations of ECRG2 (5.5, 6.5, 7.5, 8.5 µg/mL). The growth rate of the cells was found to be inhibited in a dose- and time- dependent manner by the ECRG2 protein (Figure 2).

Effects of combination treatment of ECRG2 and DDP on the EC9706/DDP cells

Based on the results above, we used 3 µg/mL of DDP and 6.5 µg/mL of ECRG2 for 48 h in EC9706/DDP cells for further experiments. As shown in Figure 3, the anti-proliferative effect of ECRG2 in combination with DDP was better compared to ECRG2 or DDP alone. These results suggest that a combination treatment of ECRG2 and DDP resulted in a synergistic decrease in cell viability compared to each treatment alone. The inhibition rate for the combination reached its peak (51.33%) at 96 h (Figure 3).

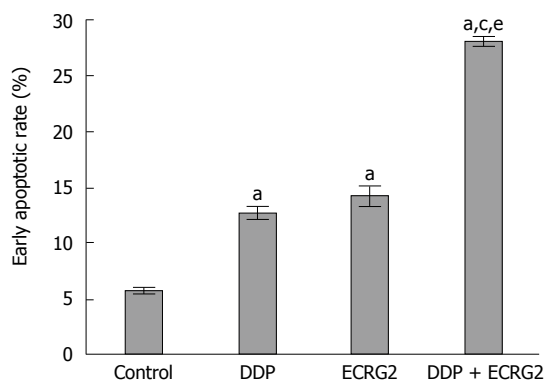
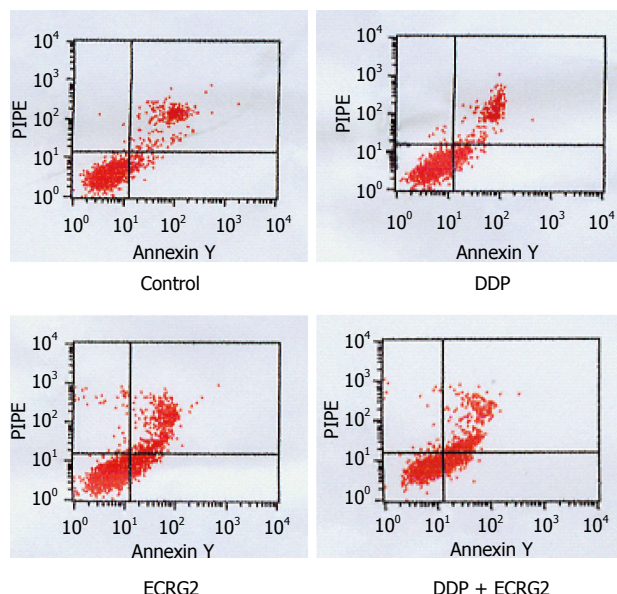


Figure 4 Effects of esophageal cancer-related gene 2 combined with cisplatin (DDP) on cell apoptosis in EC9706/DDP cells. Cells were treated with esophageal cancer-related gene 2 (ECRG2) (6.5 µg/mL) alone, DDP (3 µg/mL) alone, or a combination of ECRG2 and DDP for 24 h. Early apoptosis was determined by flow cytometry using annexin V/PI staining. Annexin V was set as the horizontal axis and PI was set as the vertical axis. Mechanically-damaged cells are located in the upper left quadrant, apoptotic or necrotic cells in the upper right quadrant, dual negative and normal cells in the lower left quadrant, and early apoptotic cells in the lower right quadrant of the flow cytometry dot plot. Data are shown as the mean ± SD. ^a*P* < 0.05 vs control group; ^b*P* < 0.05 ECRG2 + DDP vs DDP; ^c*P* < 0.05 ECRG2 + DDP vs ECRG2.

Effects of ECRG2 and DDP on apoptosis of the EC9706/DDP cells

We next examined the effect of ECRG2 and DDP on cell apoptosis by performing annexin V/PI staining. The early apoptotic rates of the control, ECRG2 alone, DDP alone, and ECRG2 plus DDP groups were 5.71% ± 0.27%, 12.68% ± 0.61%, 14.15% ± 0.87%, and 27.96% ± 0.36%, respectively. Although all treatment groups were significantly different from the control group (*P* < 0.05), the combination treatment of ECRG2 plus DDP performed significantly better when compared to either ECRG2 or DDP alone (*P* < 0.05) (Figure 4).

Effects of ECRG2 and DDP on the expression of PCNA, p53, and MT mRNA

To further elucidate the mechanisms involved in ECRG2

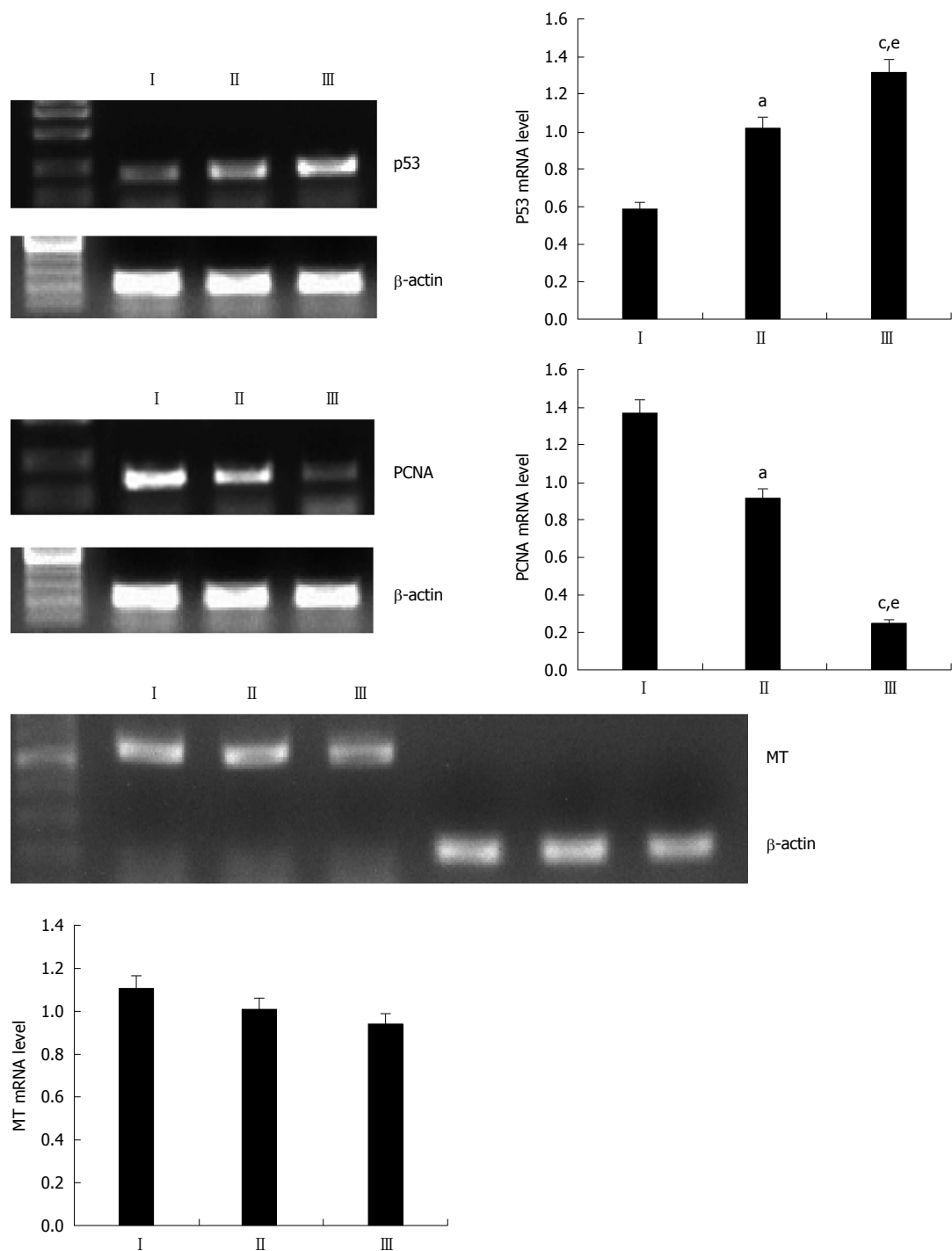


Figure 5 Effects of esophageal cancer-related gene 2 and cisplatin (DDP) on the expression of proliferating cell nuclear antigen, p53, and metallothionein mRNA. I : Drug-resistant cell group; II : DDP group; III: DDP + esophageal cancer-related gene 2 (ECRG2) group. Data were expressed as the mean ± SD, n = 3. ^aP < 0.05 vs control; ^cP < 0.05 vs control; ^eP < 0.05 vs DDP groups.

in combination with DDP on the anti-proliferative effects in drug-resistant cells, the expression of p53, PCNA, and MT mRNA were evaluated in cells using RT-PCR analysis. As shown in Figure 5, 3 μg/mL of DDP upregulated the expression of p53 and downregulated the expression of PCNA ($P < 0.05$). When ECRG2 protein was combined with DDP, p53 mRNA expression significantly increased and PCNA mRNA expression decreased compared to DDP alone ($P < 0.05$).

However, changes in the expression of MT mRNA were not observed (Figure 5).

Effects of ECRG2 and DDP on the expression of the PCNA, p53, and MT proteins

As shown in Figure 6, the results of the western blot were consistent with RT-PCR; 3 μg/mL of DDP upregulated the expression of p53 protein and downregulated the expression of PCNA protein. When

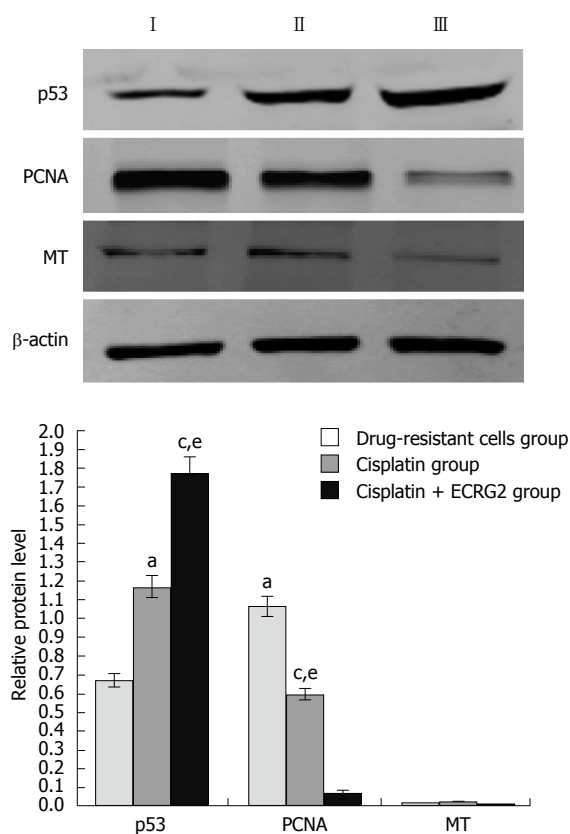


Figure 6 Effects of esophageal cancer-related gene 2 and cisplatin (DDP) on the expression of proliferating cell nuclear antigen, p53, and metallothionein proteins. I: Drug-resistant cell group; II: DDP group; III: DDP + esophageal cancer-related gene 2 (ECRG2) group. Data were expressed as the mean \pm SD, $n = 3$. ^a $P < 0.05$ vs control; ^c $P < 0.05$ vs control; ^e $P < 0.05$ vs DDP groups.

ECRG2 protein was combined with DDP, expression of p53 protein was significantly increased and expression of PCNA protein was decreased compared to DDP alone ($P < 0.05$). However, no changes were seen in the expression of MT protein (Figure 6).

DISCUSSION

DDP is one of the most effective anti-cancer agents widely used in the treatment of solid tumors, including esophageal cancer. Despite a consistent rate of initial response, DDP treatment often results in the development of chemoresistance, leading to therapeutic failure. Combination therapy has become an important method of overcoming drug resistance. There have been many attempts to overcome DDP resistance in esophageal cancer cells. Lee *et al.*^[12] reported that the novel oxygen carrier YQ23 enhanced the DDP response of chemoresistant esophageal tumor xenografts, as well as reducing tumor size and the number of animals with invasive tumors. Lai *et al.*^[13] provided evidence that 14-3-3 σ , or its related DNA repair molecule, is a promising therapeutic target for counteracting DDP resistance in esophageal squamous cell carcinoma. Furthermore, Yu *et al.*^[14] suggested

autophagy is a novel target to improve the efficiency of DDP in human esophageal cancers with acquired resistance.

In this study, we first established an EC9706/DDP cell line derived from *in vitro* treatment of sensitive cells as a model to examine the effect of ECRG2 on DDP resistance. The results demonstrated that a combination of ECRG2 and DDP had synergistic inhibitory effects on the proliferation of EC9706/DDP cells. The inhibition rate was significantly higher in EC9706/DDP cells treated with ECRG2 and DDP compared to cells treated with ECRG2 or DDP alone. This suggests ECRG2 may reduce or inhibit DDP resistance.

The mechanism through which cancer cells develop resistance to chemotherapy is associated with increased resistance to apoptosis. Agents that induce apoptosis have been reported to sensitize tumor cells to DDP^[15]. Cui *et al.*^[9,10] reported that ECRG2 promoted cell apoptosis *in vivo* and *in vitro*. Here, we provided experimental evidence showing the combination of ECRG2 and DDP promoted the apoptosis of esophageal DDP-resistant cancer cells. A study by Li *et al.*^[16] showed that ECRG2-overexpressing cells had a higher expression of p53. We therefore compared the p53 expression levels of the control, DDP alone, and ECRG2 plus DDP groups. Our results proved that p53 was involved in this process. The pivotal role of p53 pro-apoptotic function in sensitivity to DDP has also recently been reported^[17,18]. The tumor suppressor protein p53 has been shown to mediate the cellular stress response, as well as initiate DNA repair, cell-cycle arrest, senescence, and, most notably, apoptosis. However, p53 can also induce DNA-damage, thereby further promoting cell death. Therefore, we believe the combination of ECRG2 and DDP induced overexpression of p53 and then increased the response to DDP by the p53 pro-apoptotic function in esophageal DDP-resistant cancer cells.

In addition to p53, there are other molecules involved in DDP resistance. Dillehay *et al.*^[19] reported a novel small molecule targeting PCNA-induced DNA damage and apoptosis in prostate cancer cells, as well as enhancing DNA damage and apoptosis triggered by DDP in a p53-independent manner. Expression of PCNA genes in all organisms is traditionally associated with cell proliferation and thus with DNA synthesis during genome replication in the S phase of the cell cycle^[20]. However, recent developments in PCNA protein interactions, trimer formation, and signaling regulation allow for the potential of possible therapeutic targeting of PCNA^[21]. Both peptides and small molecules targeting PCNA signaling have been reported to sensitize cancer cells to chemotherapeutic agents^[22,23]. A PCNA inhibitor can increase DNA double-strand breaks and cause the accumulation of DNA damage, which will eventually lead to cell death in cells that lack the ability to repair this damage^[19]. In the present study, we demonstrated that the small

molecule ECRG2 combined with DDP reversed DDP resistance by downregulating PCNA expression in esophageal DDP-resistant cancer cells. However, the underlying molecular mechanisms of this phenomenon require further future exploration.

MT is a low molecular weight protein that is rich in cysteine^[24]. In recent years, the relationship between MT and tumors has received increasing attention, including the role of MT in tumor formation and the differentiation, proliferation, and apoptosis of tumor cells and tumor tissue in ammonia chloride DDP chemotherapy drug resistance^[25,26]. *In vitro* and *in vivo* studies show that overexpression of MT involved in DDP resistance, MT expression level, and DDP resistance level are related, with MT increasing DDP resistance^[27,28]. However, another study demonstrated that the expression of MT was not related to resistance in DDP treatment^[29-31]. In this present study, we found no significant change in the expression of MT after increasing DDP and ECRG2. ECRG2 in combination with DDP promotes cell apoptosis and inhibits cell proliferation, but not by reducing the expression of MT.

In conclusion, we showed in the present study that ECRG2 in combination with DDP reduces or inhibits DDP resistance and induces cell apoptosis by upregulating p53 expression and downregulating PCNA expression, but not MT expression. Our results suggest that the combination of ECRG2 and DDP may be a promising strategy for the clinical treatment of esophageal cancers that are resistant to DDP. The effects of combined ECRG2 and DDP treatment on DDP-resistant cancer cells should be further investigated and confirmed in other human cancer cell lines, as well as animal models of cancers.

COMMENTS

Background

Cisplatin (DDP) is one of the most effective agents in esophageal cancer, but drug resistance poses a major impediment. Combination therapy has become an important method of overcoming drug resistance. It has been reported that esophageal cancer-related gene 2 (ECRG2) inhibits tumor cell growth and promotes cell apoptosis. In our previous study, we found ECRG2, in combination with DDP, had an enhanced inhibitory effect on EC9706 cell proliferation and an increased inductive effect on EC9706 cell apoptosis. This suggests that ECRG2 may be involved in the drug resistance of esophageal cancer. Currently, there are no known studies in the literature examining the effects of ECRG2 on the drug resistance of esophageal cancer.

Research frontiers

Drug resistance and dose-limiting side effects have been a challenge to the further improvement of chemotherapy efficacy. These obstacles call for a novel combination of therapeutic approaches. Anticancer drugs may be combined with a broad spectrum of molecules such as small-molecule inhibitors, interfering RNA molecules, microRNA, oncolytic viruses, and naturally occurring substances. The ECRG2/DDP combination presents a very promising treatment approach.

Innovations and breakthroughs

This is the first known study to investigate the synergistic effect of ECRG2 in combination with DDP. In addition, the current study also explores the synergy mechanisms of ECRG2 and DDP in DDP-resistant esophageal cancer cells.

Applications

This study shows that ECRG2 in combination with DDP can inhibit viability and induce apoptosis in esophageal cancer DDP-resistant cells, possibly through upregulation of p53 expression and downregulation of PCNA expression. These findings suggest that the combination of ECRG2 and DDP may be a promising strategy for the clinical treatment of esophageal cancers that are resistant to DDP.

Peer-review

This is an interesting study about ECRG2 enhancing the anticancer effects of DDP in DDP-resistant esophageal cancer cells.

REFERENCES

- 1 **Torre LA**, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]
- 2 **Rustgi AK**, El-Serag HB. Esophageal carcinoma. *N Engl J Med* 2014; **371**: 2499-2509 [PMID: 25539106 DOI: 10.1056/NEJMra1314530]
- 3 **Kim M**, Keam B, Kim TM, Kim HG, Kim JS, Lee SS, Shin SH, Kim MK, Park KU, Kim DW, Yun HJ, Lee JS, Heo DS. Phase II Study of Irinotecan and Cisplatin Combination Chemotherapy in Metastatic, Unresectable Esophageal Cancer. *Cancer Res Treat* 2016; Epub ahead of print [PMID: 27488873 DOI: 10.4143/crt.2016.121]
- 4 **Millar J**, Scullin P, Morrison A, McClory B, Wall L, Cameron D, Philips H, Price A, Dunlop D, Eatock M. Phase II study of gemcitabine and cisplatin in locally advanced/metastatic oesophageal cancer. *Br J Cancer* 2005; **93**: 1112-1116 [PMID: 16278660 DOI: 10.1038/sj.bjc.6602842]
- 5 **Almhanna K**, Hoffe S, Strosberg J, Dinwoodie W, Meredith K, Shridhar R. Concurrent chemoradiotherapy with protracted infusion of 5-fluorouracil (5-FU) and cisplatin for locally advanced resectable esophageal cancer. *J Gastrointest Oncol* 2015; **6**: 39-44 [PMID: 25642336 DOI: 10.3978/j.issn.2078-6891.2014.101]
- 6 **Chang H**, Shin SK, Cho BC, Lee CG, Kim CB, Kim DJ, Lee JG, Hur J, Lee CY, Bae MK, Kim HR, Lee SK, Park JC, Lee H, Kim HI, Chung H, Cha J, Lee YC, Kim JH. A prospective phase II trial of S-1 and cisplatin-based chemoradiotherapy for locoregionally advanced esophageal cancer. *Cancer Chemother Pharmacol* 2014; **73**: 665-671 [PMID: 24562525 DOI: 10.1007/s00280-013-2371-y]
- 7 **Pavelic J**. Editorial: combined cancer therapy. *Curr Pharm Des* 2014; **20**: 6511-6512 [PMID: 25341927 DOI: 10.2174/1381612820666140826154834]
- 8 **Su T**, Liu H, Lu S. [Cloning and identification of cDNA fragments related to human esophageal cancer]. *Zhonghua Zhong Liu Za Zhi* 1998; **20**: 254-257 [PMID: 10920976]
- 9 **Cui YP**, Wang JB, Zhang XY, Bi MX, Guo LP, Lu SH. Using yeast two-hybrid system to identify ECRG2 associated proteins and their possible interactions with ECRG2 gene. *World J Gastroenterol* 2003; **9**: 1892-1896 [PMID: 12970870 DOI: 10.3748/wjg.v9.i9.1892]
- 10 **Cui Y**, Wang J, Zhang X, Lang R, Bi M, Guo L, Lu SH. ECRG2, a novel candidate of tumor suppressor gene in the esophageal carcinoma, interacts directly with metallothionein 2A and links to apoptosis. *Biochem Biophys Res Commun* 2003; **302**: 904-915 [PMID: 12646258 DOI: 10.1016/S0006-291X(03)00122-0]
- 11 **Song HY**, Deng XH, Yuan GY, Hou XF, Zhu ZD, Zhou L, Ren MX. Expression of bcl-2 and p53 in induction of esophageal cancer cell apoptosis by ECRG2 in combination with cisplatin. *Asian Pac J Cancer Prev* 2014; **15**: 1397-1401 [PMID: 24606472 DOI: 10.7314/APJCP.2014.15.3.1397]
- 12 **Lee NP**, Chan KT, Choi MY, Lam HY, Tung LN, Tzang FC, Han H, Lam IP, Kwok SY, Lau SH, Man C, Tong DK, Wong BL, Law S. Oxygen carrier YQ23 can enhance the chemotherapeutic drug responses of chemoresistant esophageal tumor xenografts. *Cancer Chemother Pharmacol* 2015; **76**: 1199-1207 [PMID: 26553104 DOI: 10.1007/s00280-015-2897-2]

- 13 **Lai KK**, Chan KT, Choi MY, Wang HK, Fung EY, Lam HY, Tan W, Tung LN, Tong DK, Sun RW, Lee NP, Law S. 14-3-3 σ confers cisplatin resistance in esophageal squamous cell carcinoma cells via regulating DNA repair molecules. *Tumour Biol* 2016; **37**: 2127-2136 [PMID: 26346170 DOI: 10.1007/s13277-015-4018-6]
- 14 **Yu L**, Gu C, Zhong D, Shi L, Kong Y, Zhou Z, Liu S. Induction of autophagy counteracts the anticancer effect of cisplatin in human esophageal cancer cells with acquired drug resistance. *Cancer Lett* 2014; **355**: 34-45 [PMID: 25236911 DOI: 10.1016/j.canlet.2014.09.020]
- 15 **Zhang R**, Li Y, Dong X, Peng L, Nie X. MiR-363 sensitizes cisplatin-induced apoptosis targeting in Mcl-1 in breast cancer. *Med Oncol* 2014; **31**: 347 [PMID: 25416050 DOI: 10.1007/s12032-014-0347-3]
- 16 **Li MN**, Huang G, Guo LP, Lu SH. [Inhibitory effects of esophageal cancer related gene 2 on proliferation of human esophageal cancer cell EC9706]. *Zhonghua Yi Xue Za Zhi* 2005; **85**: 2785-2788 [PMID: 16324322]
- 17 **Huang H**, Chen AY, Ye X, Li B, Rojanasakul Y, Rankin GO, Chen YC. Myricetin inhibits proliferation of cisplatin-resistant cancer cells through a p53-dependent apoptotic pathway. *Int J Oncol* 2015; **47**: 1494-1502 [PMID: 26315556 DOI: 10.3892/ijo.2015.3133]
- 18 **Li X**, Huang JM, Wang JN, Xiong XK, Yang XF, Zou F. Combination of chrysin and cisplatin promotes the apoptosis of Hep G2 cells by up-regulating p53. *Chem Biol Interact* 2015; **232**: 12-20 [PMID: 25770930 DOI: 10.1016/j.cbi.2015.03.003]
- 19 **Dillehay KL**, Lu S, Dong Z. Antitumor effects of a novel small molecule targeting PCNA chromatin association in prostate cancer. *Mol Cancer Ther* 2014; **13**: 2817-2826 [PMID: 25253786 DOI: 10.1158/1535-7163.MCT-14-0522]
- 20 **Strzalka W**, Ziemienowicz A. Proliferating cell nuclear antigen (PCNA): a key factor in DNA replication and cell cycle regulation. *Ann Bot* 2011; **107**: 1127-1140 [PMID: 21169293 DOI: 10.1093/aob/mcq243]
- 21 **Wang SC**. PCNA: a silent housekeeper or a potential therapeutic target? *Trends Pharmacol Sci* 2014; **35**: 178-186 [PMID: 24655521 DOI: 10.1016/j.tips.2014.02.004]
- 22 **Inoue A**, Kikuchi S, Hishiki A, Shao Y, Heath R, Evison BJ, Actis M, Canman CE, Hashimoto H, Fujii N. A small molecule inhibitor of monoubiquitinated Proliferating Cell Nuclear Antigen (PCNA) inhibits repair of interstrand DNA cross-link, enhances DNA double strand break, and sensitizes cancer cells to cisplatin. *J Biol Chem* 2014; **289**: 7109-7120 [PMID: 24474685 DOI: 10.1074/jbc.M113.520429]
- 23 **Yu YL**, Chou RH, Liang JH, Chang WJ, Su KJ, Tseng YJ, Huang WC, Wang SC, Hung MC. Targeting the EGFR/PCNA signaling suppresses tumor growth of triple-negative breast cancer cells with cell-penetrating PCNA peptides. *PLoS One* 2013; **8**: e61362 [PMID: 23593472 DOI: 10.1371/journal.pone.0061362]
- 24 **Mehus AA**, Muhonen WW, Garrett SH, Somji S, Sens DA, Shabb JB. Quantitation of human metallothionein isoforms: a family of small, highly conserved, cysteine-rich proteins. *Mol Cell Proteomics* 2014; **13**: 1020-1033 [PMID: 24493013 DOI: 10.1074/mcp.M113.033373]
- 25 **Tian ZQ**, Xu YZ, Zhang YF, Ma GF, He M, Wang GY. Effects of metallothionein-3 and metallothionein-1E gene transfection on proliferation, cell cycle, and apoptosis of esophageal cancer cells. *Genet Mol Res* 2013; **12**: 4595-4603 [PMID: 24222235 DOI: 10.4238/2013]
- 26 **Zamirska A**, Matusiak L, Dziegiel P, Szybejko-Machaj G, Szepietowski JC. Expression of metallothioneins in cutaneous squamous cell carcinoma and actinic keratosis. *Pathol Oncol Res* 2012; **18**: 849-855 [PMID: 22407324 DOI: 10.1007/s12253-012-9513-0]
- 27 **Kelley SL**, Basu A, Teicher BA, Hacker MP, Hamer DH, Lazo JS. Overexpression of metallothionein confers resistance to anticancer drugs. *Science* 1988; **241**: 1813-1815 [PMID: 3175622 DOI: 10.1126/science.3175622]
- 28 **Kennette W**, Collins OM, Zalups RK, Koropatnick J. Basal and zinc-induced metallothionein in resistance to cadmium, cisplatin, zinc, and tertbutyl hydroperoxide: studies using MT knockout and antisense-downregulated MT in mammalian cells. *Toxicol Sci* 2005; **88**: 602-613 [PMID: 16150881 DOI: 10.1093/toxsci/kfi318]
- 29 **Surowiak P**, Kaplenko I, Spaczyński M, Zabel M. The expression of metallothionein (MT) and proliferation intensity in ovarian cancers treated with cisplatin and paclitaxel. *Folia Morphol (Warsz)* 2003; **62**: 493-495 [PMID: 14655150]
- 30 **Tuzel E**, Yorukoglu K, Ozkara E, Kirkali Z. Association of metallothionein expression and clinical response to cisplatin based chemotherapy in testicular germ cell tumors. *Cent European J Urol* 2015; **68**: 45-50 [PMID: 25914837 DOI: 10.5173/cej.2015.01.486]
- 31 **Gansukh T**, Donizy P, Halon A, Lage H, Surowiak P. In vitro analysis of the relationships between metallothionein expression and cisplatin sensitivity of non-small cellular lung cancer cells. *Anticancer Res* 2013; **33**: 5255-5260 [PMID: 24324058]

P- Reviewer: Kantsevov S, Ong J, Tokunaga Y **S- Editor:** Qi Y
L- Editor: Rutherford A **E- Editor:** Wang CH





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>



ISSN 1007-9327



9 771007 932045