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Investigation of epigenetic modifier on HDAC1 and microRNA-410 expression in ovarian cancer cell lines

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ABSTRACT

The purpose of this study was in vitro evaluation of 5-Aza-2′-Deoxycytidine (5-Aza-dC), Trichostatin A (TSA) and Cisplatin effects on HDAC1 gene and miRNA-410 expression in ovarian cancer cell lines and comparing with cotreatment results on gene expression. Influence of 5-Aza-2′-Deoxycytidine, TSA and Cisplatin on human cells was evaluated alone and together. We measured HDAC gene and miR-410 expression by Quantitative Real Time polymerase chain reactions (qRT-PCR) and the results were analyzed by SPSS and GraphPad Prism 7 software. While each drug effects the expression of HDAC1 and miRNA-410, their coadministration had more effective results. Cisplatin, TSA, and 5-Aza-dC have a great potential in terms of therapeutic agents in ovarian cancer patients; however, more studies are needed to optimize their specificity and effectiveness.

1. Introduction

Worldwide, epithelial ovarian cancer is the leading gynecological cause of malignancy death in adult women which often presents at advanced stage with a poor prognosis. Therefore, efforts at early detection and new therapeutic approaches to reduce mortality have been largely continue. The gold standard of care for advanced ovarian cancer is preferentially a combination of platinum and taxane. However, the disease invariably progresses to a chemoresistant state, and finally, chemotherapy regimens do not provide good prognosis. Therefore, for resistant ovarian cancer, novel agents or their combinations with different cellular targets are urgently needed (Huang et al., 2016).

With the advent of the post-genome era, it has become increasingly regarded microRNAs (miRNAs) as a new regulator in numerous biological processes. miRNAs are small single-stranded endogenous RNAs (∼22 bp) that negatively regulate gene expression by degradation of messenger RNA (mRNA), through imperfect complementary pairing with 3′ untranslated region (3′UTR) of target mRNAs and inhibition of translation at the initiation or elongation. These molecules typically reduce the translation and stability of mRNAs, including those of genes that mediate processes in tumorigenesis, such as inflammation, cell cycle regulation, apoptosis, and invasion (Lu and Rothenberg, 2018).

Locating at fragile sites (FRAs) as well as loss of heterozygosity, amplification and common breakpoint regions have been raised this assumption that miRNA genes involvement in the tumorigenesis is crucial. For example, mir-15 is frequently downregulated in B cell chronic lymphocytic leukemia. More examples of association between cancer and miRNAs are decreased expression of mir-143 in colorectal cancers and let-7 in lung cancer, upregulation of mir-155 in Burkitt's lymphomas (Olson et al., 2009).

The precise mechanisms of miRNA expression are poorly understood. However, several mechanisms, including genetic alterations, may affect miRNA expression, which play a vital role target genes expression in cancers. Besides, miRNA expression patterns have relevance to the biological and clinical behavior of solid tumors such as breast cancer (Lu et al., 2005).

Indeed, previous studies have reported miRNAs as novel molecules; play vital roles in epigenetic regulation such as DNA methylation and histone deacetylation by DNA methyltransferases and histone deacetylase (HDACs) enzymes respectively. By recent reports, histone

Abbreviations: miRNAS, microRNAs; QRT-PCR, Quantitative Real Time polymerase chain; 3′ UTR, 3′ untranslated region; DNMTs, DNA Methyltransferases; HDACs, Histone deacetylase; FBS, fetal bovine serum; TSA, Trichostatin A; 5aza-cytidine, 5-aza- cytidine.

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deacetylase (HDACs) have received great attention and are considered as emerging druggable targets. HDACs are classified into four groups namely, class I (HDAC1, 2, 3, and 8), class II (HDAC4, 5, 6, 7, 9, and 10), class III (satins 1–7), and class IV (HDAC11). As HDAC modulates the acetylation status of histone and non-histone cellular proteins, they have been introduced as potential therapeutic targets for a number of human diseases (Adcock et al., 2006).

Noteworthy, miRNA-410 is located in 14q32.31 and acted as a tumor suppressor factor. It is well known that deregulated of miR-410 in various cancers, for instance the expression of miR-410 was downregulated in 75% of the breast cancer samples that seems miR-410 play an important role in tumor development and progression. In another example study Paula Mussnich research group demonstrated that miRNA-410 is also downregulated in gonadotroph adenomas compared with normal validating a critical role of miR-410 in tumor development. It is important to highlight that CCNB1 gene as target of miR-410, since its overexpression reduces CCNB1expression (a regulatory protein involved in mitosis), suggestive of miRNA-410 function also enhancing CCNB1 mRNA degradation. It is evident that CpG islands in the upstream of 18 pre-miRNAs are methylated following 5-azacytidine treatment, including miR-369, miR-615 and miR-410 (Müssnich et al., 2015; Wu et al., 2018).

Cisplatin is clinically proven to combat different types of cancers including sarcomas, cancers of soft tissue, bones, and muscles. Although such cancers have recently received better prognosis and therefore have become less life threatening; however, little is known about their cure. In addition, due to drug resistance and considerable side effects, combination therapy of cisplatin with other cancer drugs have been applied as novel therapeutic strategies for large number human cancers (Dasari and Tchounwou, 2014).

Histone acetyl transferase (HAT) and histone deacetylase (HDAC) are two classes of enzymes involved in maintaining chromatin structure through acetylation and deacetylation. An imbalance, between these two enzymes, generally leads to causes various dysfunctions, such as mitotic defects, cell growth, and malignant development. HDAC inhibitors have been explored as strong class of antitumor in a variety of cancers. Mishra et al. showed that HDAC inhibitors are able to reduce cell survival in human breast cancer cells via alteration of the human epidermal growth factor receptor 2 (HER2) (Meng et al., 2013; Hulin-Curtis et al., 2018).

Trichostatin A (TSA) is one of several inhibitors, which is increasingly introduced for therapy decision-making, especially in various types of cancer cells, when combined with radiotherapy or chemotherapy. The potent of TSA in apoptosis induction through the prohibition of cell viability, proliferation and activation of apoptosis-related proteins in a number of cancer cells, such human gastric, ovarian and small cell lung cancer cells suggesting it a promising future effective agent in cancer management (Hulin-Curtis et al., 2018).

In the current study, we applied Cisplatin, TSA, and 5-Aza-dC to evaluate their effects on ovarian human cell viability alone and together.

Furthermore, we evaluated co-administration of these agents on HDAC1 and miR-410 expression at human ovarian cell line level.

2. Material and methods

2.1. Cell culture and treatment

Human epithelial ovarian cancer cell lines; A2780S, the chemosensitive cell line and their derivative chemoresistant A2780CP were cultured in Roswell Park Memorial Institute medium (RPMI-1640) and supplemented with 10% fetal bovine serum (FBS), antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) and humidity incubated in condition of 37 °C in the presence of 5% CO2.

Chemotherapeutic drugs, 5-Aza-Cd, TSA and Cisplatin were purchased from Sigma-Aldrich (St. Louis, MO, US) and their serial dilutions from drug stock solutions for IC50 and time determination were prepared in DMSO and treated on cell lines. All stocks were stored at 4 °C and protected from light. IC50 for Cisplatin, 5-Aza-Cd and TSA obtained 1 μM, 1 μM and 0.5 μM respectively and optimal time was 24 h for all drugs. To investigate combination effects of drugs on HDAC1 gene expression different time of incubation were scheduled. Cell lines treated with drugs based on obtained IC50 of TSA and variable dose of 5-Aza-Cd and Cisplatin in optimal time.

2.2. RNA isolation and real-time reverse transcription-quantitative polymerase chain reaction

In this study RNA extracted from treated cell lines and instantly cDNA was synthesized for gene expression. cDNA synthesis and qRT-PCR performed with two separated protocols for mRNAs and microRNA.

2.3. MRNAs gene expression

Total mRNA from each cell line was extracted using RNeasy Minikit (Qiagen, Inc.) according to the manufacturer's instructions. RNA quality was examined using A260/A280 readings. Complementary DNA (cDNA) was synthesized by cDNA synthesis kit (Taqman Reverse Transcription Reagents (Thermo Scientific)) from 1 μg of total RNA. CDNA was diluted at a ratio of 1:20 with polymerase chain reaction (PCR) grade water and then stored at −20 °C. Following on, qRT-PCR assays of HDAC1 and GAPDH cDNA performed using the SYBR Green kit (Qiagen, Inc.) in a Corbet Sequence Detection System (Qiagen).

Each PCR was performed in triplicate by using 5 μL 2× SYBR Green PCR master mix, 0.2 μL of primer sets, 1 μL cDNA, and 3.6 μL nucleotide-free H_2O to obtain a yield of 10 μL per reaction. Expression rates were calculated as the normalized C_T difference between different samples after adjusting for amplification efficiency relative to the expression level of human GAPDH gene as an internal control, and normalized for HDAC1. The specific primers used for RT-PCR are listed in Table 1.

2.4. Investigations on microRNA expression

For investigation of microRNA expression cDNA syntheses from total RNA that were extracted with Qiagen kit and qPCR assay were performed according to manufacturer's instructions (Parsgenom Company, Tehran, Iran). The main protocol had the following details:

Preparation a mixture including; 2 μg of RNA, poly A enzyme, buffer and a 10 min incubation time in 37 °C was prepared for attachment of poly A to microRNAs. Subsequently, RNA poly A tail was mixed with microRNA cDNA synthesis specific primer and reverse transcriptase enzyme which was followed by incubation at 45 °C for 60 min. The SYBR Green qRT-PCR reaction for detection and quantification of the target was performed with 4 pmol of forward primers, 8 pmol of Cy5-labeled reverse primer, 10 μL of 2× SYBR Green I Master Mix (Roche Diagnostics, Mannheim, Germany).

The following thermal profile was used for PCR amplification of 500 ng of genomic DNA on a GeneAmp PCR system 9700 (Applied Biosystems); an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of 95 °C for 5 s, 64 °C for 30 s, and 72 °C for 30 s. U6 was used as an endogenous control to normalize the amount of mir-410 transcript. We also investigated miR-410 expression after am TSA, 5-Aza Cd, TSA, and 5-Aza Cd TSA mixture in a specific time course (Fig. 3).

All the materials and procedures used are approved by the office of

Table 1
Primer list.

mRNA gene	Primer sequence
GAPDH forward	5′TGCACCACCAACTGCTTAGC 3′
GAPDH reverse	5′GGCATGGACTGTGGTCATGAG3′
HDAC1 forward	5′CCAAGTACCACAGCGATGAC 3′
HDAC1 reverse	5′TGGACAGTCCTCACCAACG 3′

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2.5. Statistical analyses

All data were double-entered, cross-checked for consistency, and analyzed using the Statistical Package for Social Sciences version 17 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.01. Additionally, relative mRNA expression was calculated by $2^{-\Delta\Delta CT}$ method. Significant differences among groups were determined using unpaired Student's *t*-test. A value of $P < 0.05$ was considered as an indication of statistical significance.

3. Results

3.1. 24 h was optimum time for obtaining an effective IC50

We firstly measured cell viability of ovarian cancer cell lines of TSA, 5-Aza-Cd, cisplatin and combined drugs.

In order to investigate IC50 the different concentrations (from 0.01 μM to 100 μM) of agents were applied on both cell lines 24 h (Fig. 1A and B). We found following IC50 for TSA, 5-Aza-Cd, and Cisplatin 0.5, 1 and 1 μM respectively.

Combine treatment including evaluation constant dose of Cisplatin (1 μM) and 5-Aza-2′-Deoxycytidine (1 μM), and variable dose of TSA (0.5, 0.75, 1 μM) was administrated simultaneously (Fig. 1C). This experiment done on A2780S cell line only. Indeed, suppression of cell viability was enhanced by cotreatment and Coefficient of Drug Interaction (CDI) was less than one that; shows synergistic effect between drugs.

3.2. Time course with effective IC50 for obtaining best time for treatment

We also studied whether different time period (16, 24, 48, 72 h) would contribute to the anti-viability effects of drugs. Each cell lines treated with cisplatin, 5Aza-Cd and TSA in different times. The results indicated that in 24 h is the best time for the maximal reduction in cell number. Fig. 2A and B shows analysis data by prism software on A2780S and A2780CP respectively.

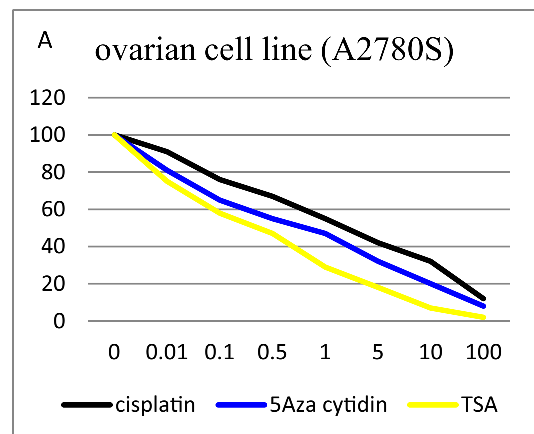
3.3. Investigation of HDAC1 expression in treated cell lines

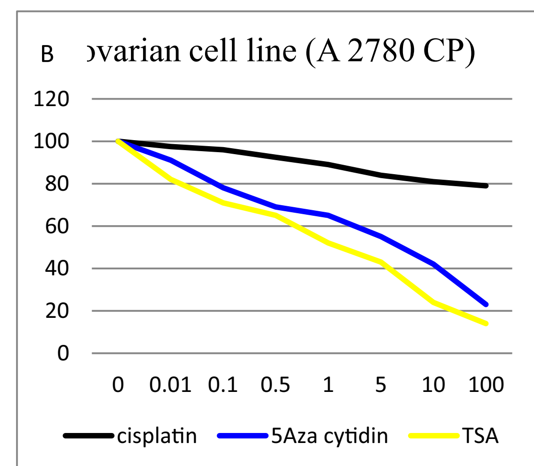
To study the potentially inhibitory epigenetic role of above drugs on HDAC1 gene expression, we examined the expression level of it on A2780s and A2780cp ovarian cancer cell lines after using Cisplatin (1 μM), TSA (0.5 μM) and Cisplatin/TSA (1 μM/0.5 μM) following incubation time in 37 °C for 24 h. There was a reduction of HDAC1 gene expression in both cell lines. While treatment with TSA/cisplatin showed a moderate reduction in HDAC1 expression, using cisplatin and TSA separately revealed a mild reduction of it (Fig. 3A, B). These results support the idea that injection of mixed therapy might be more accurately compared with single treatment. While reduced expression was clear in different time period 72 h exposure had the most effective effect (Fig. 3C, D).

3.4. miR-410expression analysis in treated cell lines with cisplatin, TSA and 5-Aza-dC

The effects of TSA, 5-Aza Cytidine and Cisplatin investigated on miR-410 expression in A2780S and A2780CP ovarian cancer cell lines.

We examined whether these drugs have any effects on miR-410 after treatment of A2780s and A2780cp ovarian cancer cell lines with Cisplatin (1 μM), TSA (0.5 μM), 5-Aza-Cytidine (1 μM), Cisplatin/TSA (1 μM/0.5 μM) and Cisplatin/5-Aza-Cytidine (1 μM/1 μM) following incubation time of 24 h in 37 C that result showed in Fig. 4A and B. in other experiment cells treated with TSA and cisplatin and 5-Aza-Cd add in different incubation time (24, 48, 72). As presented in Fig. 4C and D,





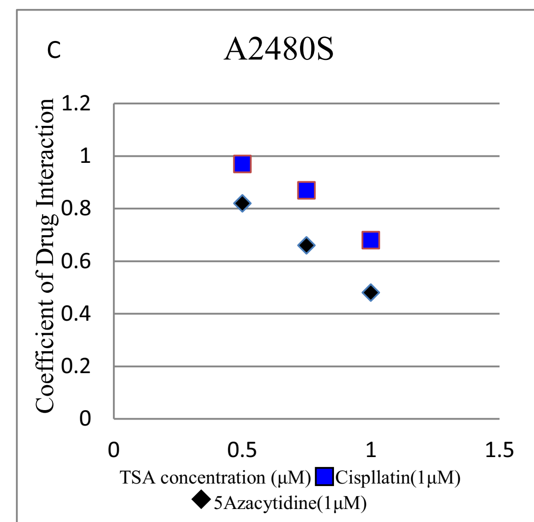


Fig. 1. Analyses of the cell viability of human ovarian cancer cell line treated with cisplatin, 5-aza-cytidine and TSA. (A) Graphing dose-response curves of drugs studied under treatment with (10^{-2} to 10^{2} μM) for 24 h. IC50 of cisplatin, 5-aza-cytidin and TSA was determined 1, 0.5 and 1 μM respectively on A2780S cell line. (B) Dose response curve of drug treatment on A2780CP cell line. Cisplatin hadn't effect on A2780CP. (C) Results of triplicate cotreatment on A2780s cells with CDI < 1 as synergism effect. Dose-response curves of drugs studied under co- treatment. Cisplatin was 1 μM.

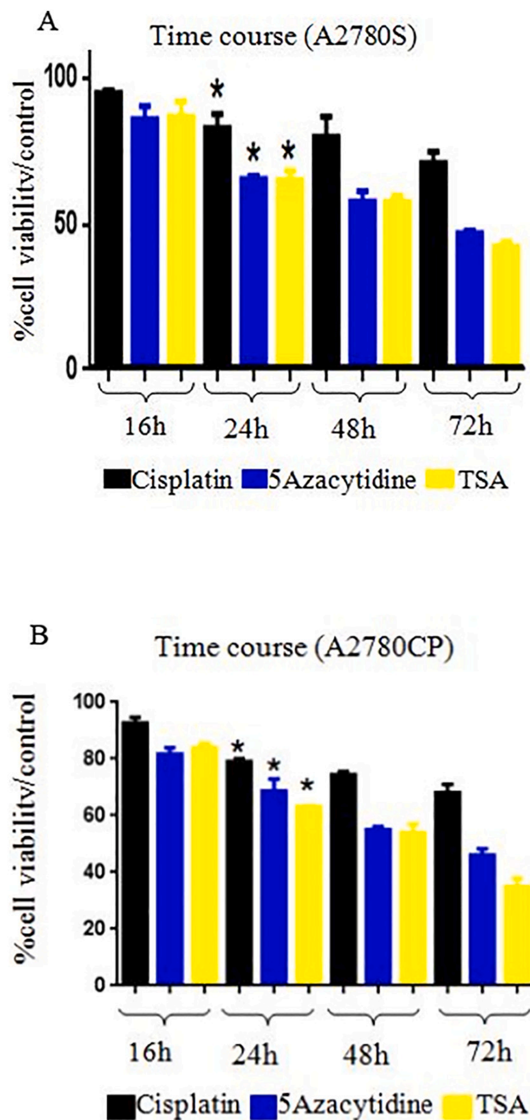


Fig. 2. Time course analysis of cell viability of human ovarian cancer treated with cisplatin, 5-aza-cytidine and TSA. (A) Time course graphing of drugs studied under treatment with IC50 for different time 16, 24, 48 and 72 h. 24 h was optimal time on A2780S cell line for all drugs treatment. (B) Treatment on A2780CP shows 24 h was optimal time. Analysis done with prism software by student's *t*-test and P value<0.05.

miR-410 significantly upregulated in a dose-dependent manner with the best effectiveness at 72 h. To quantify miRNA expression levels, U6 was used as an internal control. These results were analyzed by SPSS and GraphPad Prism 6.01 software. ($P < 0.05$).

4. Discussion

Increasing evidence has been shown that miRNAs function as vital players and therapeutic targets in different human cancers, including ovarian cancer. The expression of miRNAs is remarkably deregulated in ovarian cancer indicating the involvement of miRNAs in the onset and progression of it (Corney and Nikitin, 2008).

Epigenetics is defined as heritable alterations in gene expression that are not due to changes in the DNA sequence. Epigenetic deregulation are a hallmark of cancer in both the onset and progression which are potentially reversible suggesting promising therapeutic targets for reversing a malignant cell population to a normal one. The main investigated process of epigenetic deregulation is DNA methylation, noncoding RNAs (ncRNAs) and histone modifications which affect transcription, transcription and translation respectively. Epigenetic regulation including DNA methylation and histone deacetylation are frequently associated with transcriptional suppression of gene expression and with reduced responsiveness to chemotherapy. DNA hypermethylation and histone deacetylation are regarded as a main ovarian cancer chemoresistance by amplification of key cell survival proteins (Lu and Rothenberg, 2018).

Indeed, ovarian cancer is associated with overexpression DNMTs and HDACs and upregulation of DNMT1 and HDAC1 is strong in advanced ovarian tumors. Furthermore, relevance of promoter methylation for miRNA expression has been shown in cell lines. The first evidence of miRNA role in human cancer was reported in chronic lymphocytic leukemia (CLL) when that miR-15 and miR-16 were deleted or downregulated in about 73% of the CLL cases. Deregulation of miRNAs has been characterized in the development other human cancers and to relate with prognosis outcome, or treatment outcome in various cancers. These findings highlight the importance of miRNAs in diagnostic and/or prognostic biomarkers of malignancies (Kwon and Shin, 2011).

As mentioned the clinical response rate to chemotherapy with surgery in the late stages of ovarian cancer is satisfactory, but the repetitive relapse and acquired chemoresistance are obstacle in management of ovarian cancer which its pathway is largely unknown. This provides the urgent need to determine the related molecular mechanism to investigate a novel therapeutic target (Fraser et al., 2003).

Extrinsic chemoresistance promotes secondary to genetic and epigenetic variation in cell apoptosis, DNA repair after the administration of repetitive cycles of chemotherapy. Genetic changes is considered to the changes in the DNA sequence, including mutation, deletion, duplication, and translocation, while epigenetic alterations such as DNA methylation, histone modifications and miRNA regulation. The deregulation of miRNAs alters a combination of functional targets and signaling pathways, leads to acquired chemoresistance in human cancers. Among those, for instance overexpression of miR-21 causes acquired trastuzumab resistance by suppressing PTEN expression during long-term exposure in breast cancer, and the repetitive administration of cisplatin to A549 lung cancer cells induces an increase in miR-630, which arguments Carboplatin by inhibiting the p53 signaling pathway. These finding encouraged us to hypothesize that some miRNAs may be involved in a tumor setting the phenotypic effect developing acquired chemoresistance in ovarian cancer progression. Moreover, histone deacetylation is a prominent histone modification can be regulated chromatin structure modification and gene expression that involved in cell cycle checkpoints, DNA repair and apoptosis (Magee et al., 2015).

TSA can regulate gene expression by modulating the chromatin structure. It is known to induce growth arrest and apoptosis in cancer cells. TSA is also an antifungal antibiotic and a specific inhibitor of HDAC activity. It has a tubular structure with a zinc atom at its base and it fits into this structure with the hydroxamic moiety of the inhibitor binding to the zinc. TSA can regulate gene transcription by changing the histone acetylation level (Khajehnoori et al., 2020).

Our study showed that TSA could induce apoptosis of cells on human epithelial ovarian cancer cell lines; A2780s, the chemosensitive cell line and their derivative chemoresistant A2780cp cell line in different concentrations and exposure time which 24 h time clearly shown the most effective outcome of IC50 at 0.5 μM concentration. Our results were consistent with previous studies, which demonstrated that TSA induced apoptosis by DNA fragmentation in a concentration-dependent manner by increased chromatin relaxation.

Next, we tested whether DAC and Cisplatin treatment affects viability and colony formation of the included cell lines. As shown in Fig. 1 treatment with DAC and Cisplatin reduced cell viability of all tested cell lines. Induction of apoptosis could already be observed after treating the cell lines with various concentrations (from 0.01 μM to 100 μM); however, stronger effects were obtained by 1.0 μM. Proliferation

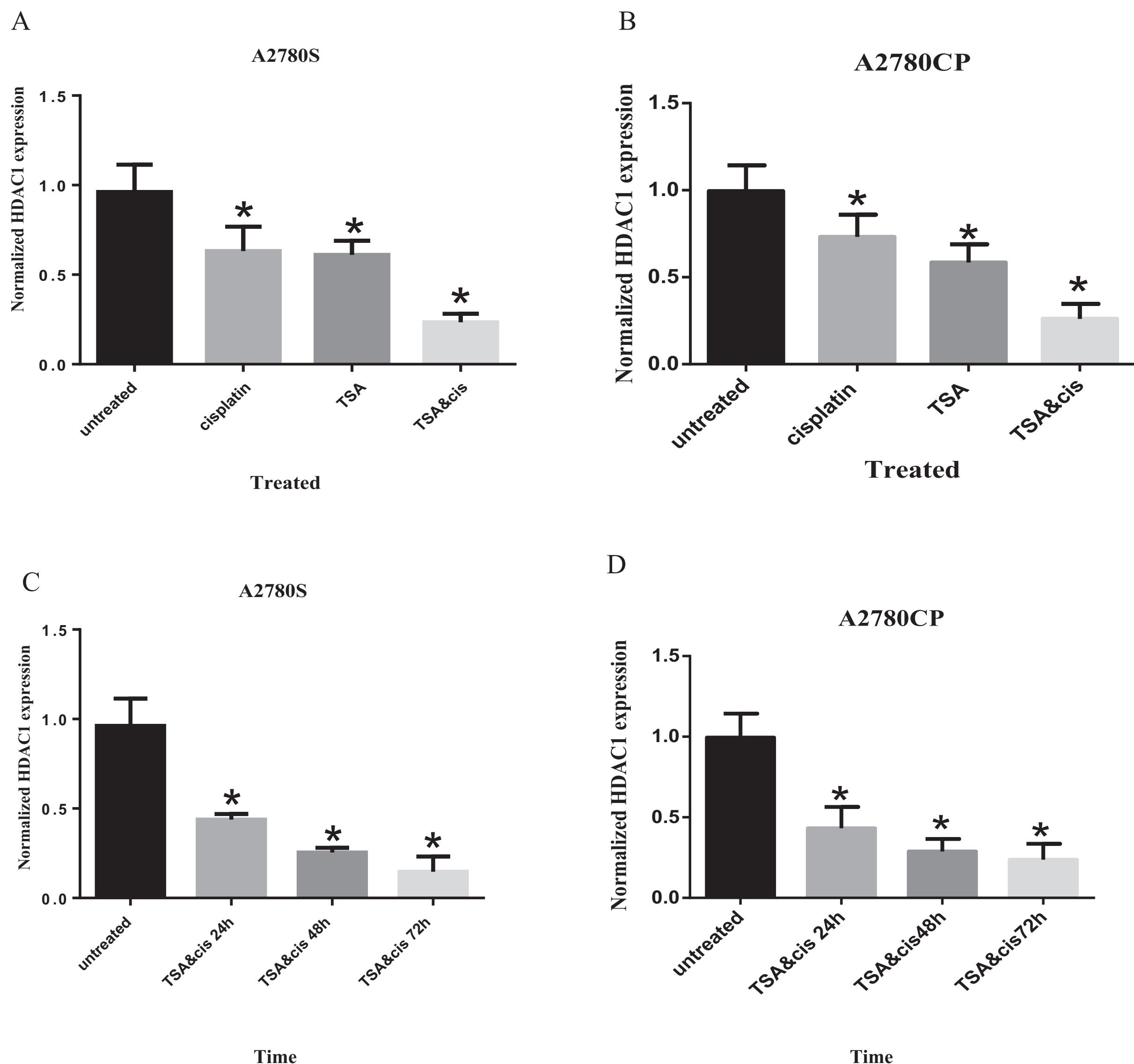


Fig. 3. Evaluation of the expression of HDAC1 by qRT-PCR in duplicate manner which each test was repeated twice. Panels A and B show the relative expression level of it was normalized by GAPDH on A2780S and A2780CP cell line respectively. Each bar represents the change in HDAC1 expression of according to cisplatin, TSA and cisplatin/TSA usage showed further confirmation for the importance of co-treatment. Panels C and D show TSA and cisplatin co-treatment at different time (24, 48 and 72 h). Cell lines treated with TSA at first and after certain time cisplatin added to culture. The results were analyzed by SPSS and GraphPad Prism 6.01 software. ($P < 0.05$).

was most effectively reduced in 24 h time period.

The next series of experiments addressed the question of whether there is a synergistic effect of combined agents on ovarian cell cytotoxicity. Therefore, cells were incubated with different TSA (from 10–2 μM to 100 μM), for 24 h, 48 h, and 72 h either alone or in combination. The viability of cells co-incubated with them decreased by 50% (IC50), compared to a 20%–30% decrease in cells treated with either TSA or DAC alone. These results indicated a synergistic effect between TSA, DAC, and on HeLa cell cytotoxicity. Moreover, the lower concentrations of these compounds would reduce the side effects; therefore, it is a better potential therapeutic approach. It could be a novel and effective tool to kill cancer cells effectively in a time and dose-dependent manner. Cecconi et al. demonstrated a show that the combined treatment of pancreatic endocrine tumor cell lines with TSA and the DNA methyltransferase inhibitor DAC determines a strong synergistic inhibition of proliferation mainly due to apoptotic cell death. Additionally, our results also were consistent with previous studies, which demonstrated that effect of combined 5-aza-2'deoxycytidine and cisplatin treatment on the P15 lung adenocarcinoma cell line suppress the growth of P15 tumor cells in comparison with DAC or CDDP treatment alone (Cecconi et al., 2009).

Kim et al. demonstrated that TSA as a HDAC1 inhibitor and in a variety of human cell line enhanced sensitivity TSA as a HDAC1 inhibitor can inhibit the growth of cancer cells and introduce TSA as a new target for therapy of lung cancer. As regards HDAC1 and DNMT1 contribute to gene silencing through recruiting transcriptional repressors to promoter regions (Kim et al., 2003).

Ercan Cacan et al. identify HDAC1 and DNMT1 as two important epigenetic regulators, that HDAC1 knockdown increases cisplatin stimulated apoptosis in chemoresistant ovarian cell lines. In this study investigated HDAC1 expression when used cisplatin and two epigenetic drugs 5-aza-cytidine and TSA. Our data support inhibition of TSA as a therapeutic approach to increase gene HDAC1 expression and overcome ovarian cancer chemoresistance (Cacan et al., 2014).

HAT and HDAC activity can be altered by mutation, overexpression, or translocation, disrupting the acetylation-deacetylation balance and

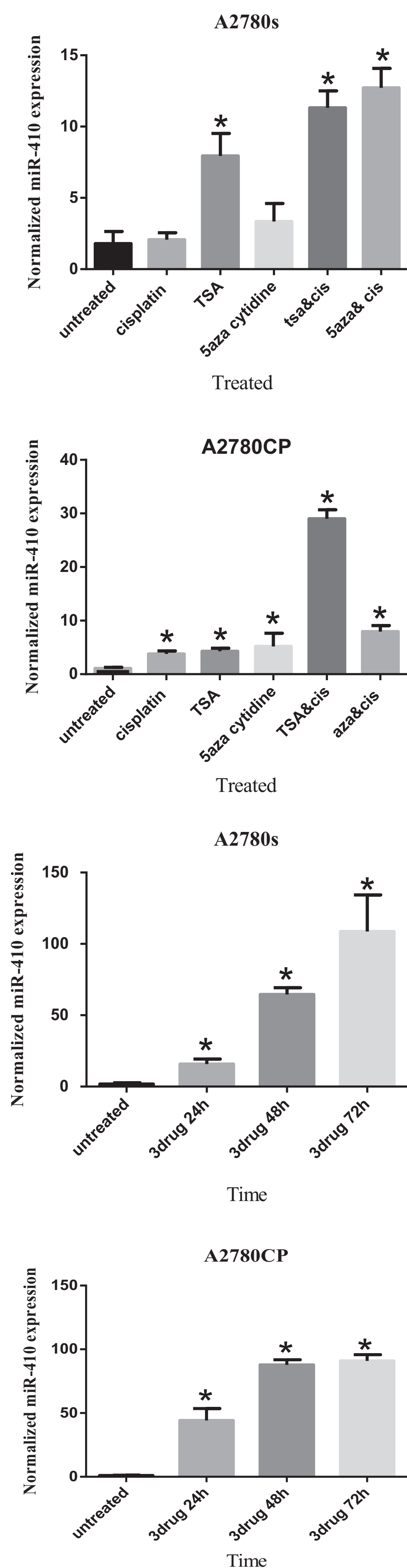


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Fig. 4. Duplicate Real Time PCR results for miR-410 expression. After treatment with Cisplatin, 5-Aza-Cytidine and TSA their combination on ovarian cancer cell showed overexpression of miR-410 (A). A2780s and (B) A2780cp. MiR-410 expression measured after cell line treated by TSA 24, 48 and 72 h following adding Cisplatin and 5-Aza-cytidine for 24 h. (C) A2780s, (D) A2780cp. The variation on the results was statistically significant. ($P < 0.05$).

consequently contributing to cancer hallmarks; these epigenetic changes have been observed in leukemia and prostate, breast, colorectal, and ovarian cancers. Acetylation changes are thought to participate in carcinogenesis by silencing tumor suppressor gene promoters, activation of repressed genes, or abnormal recruitment of HATs or HDACs. It has previously been shown that HDAC1, HDAC3, and HDAC4 might be associated with resistance to chemotherapy and poor prognosis in cancer patients (Hagelkruys et al., 2011).

Previous reports have revealed the efficacy of combined HDAC inhibitor and cytotoxic chemotherapy for instance romidepsin (FK228), approved for phase I and II trials, improves the cytotoxic effects of cisplatin through inhibiting of cell growth and inducing apoptosis. Or HDAC2 depletion reduced the IC50 of cisplatin in PEO1 cells, suggesting that HDAC2 suppression enhances the cisplatin efficacy (Huang et al., 2016).

We next investigated to determine whether pharmacologic inhibitors of histone deacetylation and DNA methylation can influence the expression of HDAC in chemoresistant ovarian cancer cells. TSA, 5-Aza-Dc and Cisplatin were applied to prohibit HDAC. To explore potential synergistic roles of drugs combination studies were performed in ovarian cancer cell lines. Again, TSA, 5-Aza-dC and Cisplatin alone suppressed HDAC expression in A2780s and A2780cp ovarian cancer cells, and the combination of these drugs results in a fold reduction in HDAC expression greater than the sum of the individual effect, suggesting a potential cooperative effect (Fig. 2A). While treatment with TSA/cisplatin showed a moderate reduction in HDAC1 expression, using cisplatin and TSA separately revealed a mild reduction of it. Our study supports recent reports indicate tumorigenicity and metastasis of ovarian cancer cells is significantly suppressed by the combination of HDAC inhibitor TSA and 5-Aza-dC.

A recent phase I clinical trial was the first to attempt to reverse platinum resistance in ovarian cancer with a combination of methylation and histone deacetylase inhibition. These results show that changing methylation and acetylation results in changes in clinical outcomes. Further, preselecting patients based on known methylation status may optimize treatment responses. Our study supports HDAC genes as targets for ovarian treatment (Bandolik et al., 2019).

Our finding here supports this idea that miR-410 is target of HDAC in A2780s and A2780cp ovarian cancer cells. Moreover, our data supports that a reduction in miR-410 expression involves ovarian cancer cell death. To our knowledge, this is the first study showing a regulatory role for HDAC in ovarian cancer cell lines through upregulation of mir410. Based on these results TSA, 5-Aza-dC and Cisplatin treatment are able to suppress ovarian cell tumor growth in vitro, suggesting their therapeutic potential for patients. Most importantly, we provide evidence that the miR-410/HDAC signaling pathway is deregulated in human ovarian cancer.

As reported the role of miR-410 in different types of cancer. Chein et al. published their results that Chien et miR-410 negatively regulates pRb/E2Fpathway by repressing CDK1 oncogene in breast cancer. Plus, Gattoliat et al. group has demonstrated that miR-410 expression is significantly associated with disease free survival of neuroblastoma. Considering that miR-410 has a reduction expression in a panel of prostate cancer cell lines. The current study is the first one that identifies miR-410 as a major regulator of ovarian growth showing both functional importance and therapeutic potential.

5. Conclusion

Overall, Cisplatin, TSA and 5-Aza-dC have a great potential in terms of therapeutic agents in ovarian cancer patients, however additional studies are needed in order to optimize their specificity and effectiveness, minimizing their off-target effects.

Chemo-resistance during tumor progression is the major obstacle of clinical management of ovarian cancer that its underlying mechanisms are poorly understood. Here, we identified that blocking HDAC and overexpression of miR-410 by using Cisplatin, TSA and 5-Aza-dC alone or in combination dramatically abrogated tumor growth and enhance in vitro in ovarian cancer cells.

Our study provides a novel regulatory circuit in which the epigenetic machinery silences the expression of miRNAs to deregulate the activity of the key signaling pathways involved in developing ovarian cancer chemoresistance. Therefore, targeting the miR-410 and HDAC using Cisplatin, TSA and 5-Aza-dC regulatory circuit might be a novel therapeutic approach in chemoresistant ovarian cancer.

Declaration of competing interest

We have no conflict of interest to declare.

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