RESEARCH ARTICLE

3-Deazaneplanocin A is a Promising Therapeutic Agent for Ovarian Cancer Cells

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Abstract

Background: Recent studies have shown that 3-deazaneplanocin A (DZNep), a well-known histone methyltransferase inhibitor, disrupts polycomb-repressive complex 2 (PRC2), and induces apoptosis, while inhibiting proliferation and metastasis, in cancer cells, including acute myeloid leukemia, breast cancer and glioblastoma. However, little is known about effects of DZNep on ovarian cancer cells. Materials and Methods: We here therefore studied DZNep-treated A2780 ovarian cancer cells in vitro. Proliferation of ovarian cancer cells under treatment of DZNep was assessed by MTT and apoptosis by flow cytometry. Cell wound healing was applied to detect the migration. Finally, we used q-PCR to assess the migration-related gene, E-cadherin. Results: DZNep could inhibit the proliferation of A2780 and induce apoptosis Furthermore, it inhibited migration and increased the expression of E-cadherin ($P<0.05$). Conclusion: DZNep is a promising therapeutic agent for ovarian cancer cells, with potential to inhibite proliferation, induce apoptosis and decrease migration.

Keywords: DZNep - ovarian cancer cells - proliferation - apoptosis - migration

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Introduction

Ovarian cancer is the most lethal gynecological malignancy (Bristow et al., 2010). The high mortality rate of ovarian cancer is because of poor prognosis and most cases are detected at late stage. Currently the treatment of ovarian cancer is generally combination chemotherapy with a platinum agent and a taxol. However, with this regimen, many patients experience relapse of their cancer and development of drug resistance is also not uncommon, making successful second line therapy very difficult to achieve.

The enhancer of Zeste homologue 2 (EZH2) encodes a histone methyltransferase, which is the catalytic core protein of the polycomb repressor complex 2 (PRC2) (Cao et al., 2002; Kuzmichev et al., 2002). PRC2 is well known as initiating target gene silencing by promoting H3-K27 trimethylation, which is catalyzed by EZH2. Guo and colleagues found that EZH2 was overexpressed in ovarian tumor with the highest level expression in malignant ovarian tissues, and the variation of EZH2 expression at different pathological type/grade and International Federation of Gynecology and Obstetrics (FIGO) stages was statistically significant (Guo et al., 2011). Rizzo and colleagues found a key role for EZH2 in the maintenance of a drug-resistant, tumor-sustaining subpopulation of cells in ovarian cancers undergoing chemotherapy (Rizzo et al., 2011).

3-Deazaneplanocin A (DZNep), is a potent inhibitor of S-adenosylhomocysteine (AdoHcy) hydrolase (Glazer et al., 1986; Liu et al., 1992). Inhibiting AdoHcy hydorlase results in accumulation of AdoHcy, which leads to product inhibition of S-adenosyl-L-methionine-dependent methyltransferases. This indirectly inhibits methyltransferase activity by limiting available methyl donor groups. DZNep appears to be a unique chromatin-remodeling compound that can deplete cellular PRC2 protein (Tan et al., 2007; Kalushkova et al., 2010; Kikuchi et al., 2012). The potential mechanisms for the therapeutic effects of DZNep on cancer cells include proliferation inhibition, angiogenesis inhibition, and the induction of apoptosis.

However, very little is known on its effects of DZNep on ovarian cancer cells. In our present study, we therefore aimed to examine whether DZNep has proliferation-attenuating and apoptosis-inducing effects on ovarian cancer cells.

Materials and Methods

Cell culture and treatment

Human ovarian cancer cell line A2780 were maintained in RPMI 1640, supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/mL streptomycin. A2780 cells were grown at 37°C in a humidified atmosphere with 5% CO_2. DZNep were order from Cayman chemical. DZNep was dissolved in DMSO (Sigma) at 10 mmol/L and stored as frozen at -20°C.

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Proliferation study

For cell viability assays, cells were plated in 96-well dishes (1×10^4 cells/well) and the next day were treated with or without DZNep in RPMI 1640 and grown over 48h and 72h period. Cell viability was assessed using Cell Proliferation Kit I (Roche) according to the manufacturer's instruction.

Apoptosis analysis

Cells were washed twice in phosphate-buffered saline and then stained with Annexin V-FITC (Roche) and 7-AAD according to the manufacturer's instructions. After staining with Annexin V-FITC and 7-AAD, samples were analyzed by fluorescence-activated cell scanner (FACScan) flow cytometer.

Cell migration assays

The effects of DZNep on migration of A2780 were tested using wound-healing assay. Briefly, cells were grown in 6-well plates at a density of 5×10^4 cells/well and were incubated for 24h. Then monolayers were scratched horizontally with a pipette tip to obtain a monolayer culture with space without cells. Media and dislodged cells were aspirated and the plates were washed twice with PBS, then fresh RPMI 1640 containing DZNep (5 μM), and incubation continued for 48h. Three randomly selected fields along the scraped line were photographed on each well at the time of the wounding and at 48h intervals thereafter using OLYMPUS inverted micro-scope. Migration was estimated by calculating the migration distance of cell interfaces.

RNA extraction and real-time PCR analysis

A2780 were exposed to 0 and 5 μM DZNep for 48 h. The mRNA of A2780 was isolated using Trizol reagent (Invitrogen, CA) and reverse transcribed into cDNA. Quantitation of the relative EZH2 and E-cadherin mRNA abundance was performed using an Applied biosystems viia7 (Applied Biosystems). The glyceraldehyde-3 phosphate dehydrogenase (GAPDH) gene served as an internal control. Differences in threshold cycles between the target genes and the housekeeping gene (GAPDH) were calculated. The value of the relative mRNA quantity for the control group was arbitrarily set to one for normalization.

Statistical analysis

Windows SPSS v.16.0 (SPSS, Chicago, IL) was used to analyze the data. All data were expressed as the mean±SD. Differences between groups were analyzed by one-way analysis of variance (ANOVA). *P* value<0.05 was considered statistically significant.

Results

DZNep reduces the proliferation of A2780

To determine the effect of DZNep on proliferation of A2780, A2780 were incubated with various concentrations of DZNep for 48h and 72h. As shown in Figure 1, we could find that DZNep could significantly inhibit proliferation at 2μM, 4μM, 6μM, 8μM and 10μM.

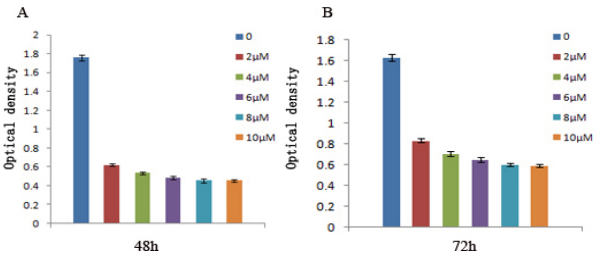

Figure 1. DZNep Inhibits Proliferation of A2780. (A) Treatment of several different concentrations of DZNep on A2780 for 48h. (B) Treatment of several different concentrations of DZNep on A2780 for 72h

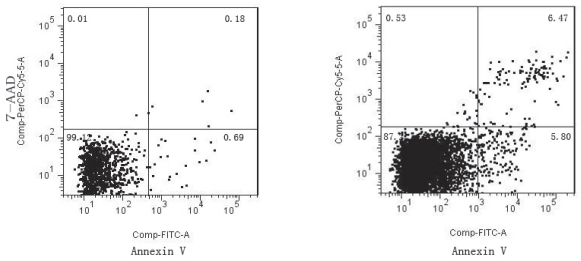

Figure 2. DZNep Induces Apoptosis on A2780. A2780 cells were treated with 5 μM DZNep for 48h. Following treatment, cells were stained for Annexin, a cell surface marker of apoptosis. DZNep significantly induced apoptosis in DZNep -treated cells ($P < 0.05$)

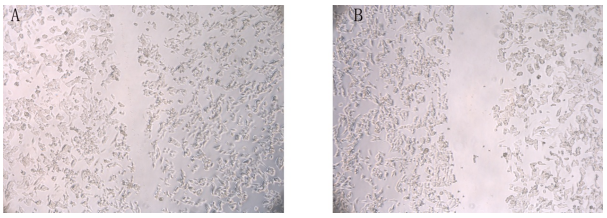

Figure 3. DZNep Inhibits Migration of A2780. In wound-healing assay, after scratched wound, cells monolayers were incubated with DZNep for 48 h. Images of wounded monolayers were taken at times 48 h after treatment with control (A) and DZNep (B)

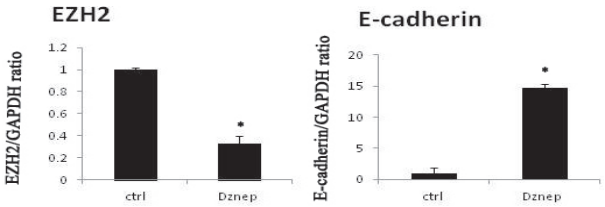

Figure 4. DZNep Enhances the mRNA Expression of E-Cadherin. (A) DZNep inhibits expression of EZH2. (B) DZNep increases expression of E-cadherin (*P<0.05)

DZNep induces apoptosis in A2780

We analyzed the number of apoptotic cells after treatment by flow cytometry using Annexin-V-FITC and 7-AAD staining (Figure 2). The results revealed that the DZNep-treated A2780 produced an induction of apoptosis.

DZNep inhibits migration by increasing the expression of E-cadherin in A2780

The effects of DZNep on migration of A2780 were examined with wound-healing assay. Monolayers cultures with space without cells were incubated with DZNep (5μM), and cell migration over the following 48h were observed. Figure 3A and B shows that there was little

migration measured in the control group, whereas in the DZNep group, cell migration was significantly inhibited ($P<0.05$). To explore the mechanism of DZNep on migration, we use the q-PCR to test the expression of E-cadherin, a migration related gene. We found that compared with control, DZNep could significantly increase the expression of E-cadherin (Figure 4).

Discussion

Our experiments demonstrated for the first time that DZNep could reduce proliferation, induce apoptosis and inhibit migration in A2780. Furthermore, we found that DZNep inhibited the expression of the E-cadherin. These observations provide the basis for further investigation of the cellular and molecular mechanisms of DZNep in the treatment of ovarian cancer.

Several evidences indicated that EZH2 is involved with invasion and metastasis in ovarian carcinoma. Rao found that high expression of EZH2 was found in none of the normal ovaries, in 3% of cystadenomas, in 23% of borderline tumors and in 50% of the ovarian carcinomas, respectively (Rao et al., 2010). Drug resistance is a major obstacle for ovarian cancer therapy. Rizzo and colleagues found that an important role for EZH2 in the maintenance of a drug-resistant, tumor-sustaining subpopulation of cells in ovarian cancers undergoing chemotherapy (Rizzo et al., 2011).

We examined the proliferation of ovarian cancer cell line to DZNep, an inhibitor of S-adenosylhomocysteine hydrolase, which has recently been shown to decrease the expression of EZH2. Our data are in agreement with previous reports demonstrating that DZNep treatment could inhibit the proliferation of many cell types (Tan et al., 2007; Sasaki et al., 2011).

Apoptosis, is a highly regulated cellular process between cell proliferation and cell death (Orrenius et al., 2003; Danial and Korsmeyer, 2004). Loss of function of the apoptosis may promote the proliferation of abnormal cells and eventually lead to cancer. In agreement with its inhibitory effects on inhibiting cell viability and proliferation, we found that treatment with 5μM DZNep for 48 h drastically increased A2780 apoptosis in vitro.

Cell migration is a very important progression involved in the metastasis of ovarian cancer. Cancer is associated with abrogation of the normal controls that limit cell migration and invasion, leading eventually to metastasis. In this context, we demonstrated that DZNep induced an inhibition of cell migration in vitro. To elucidate the mechanism of DZNep-inhibited migration in A2780, we examined the expression of migration related E-cadherin. E-cadherin is a cell adhesion protein, which plays a major role in maintaining intercellular junction in epithelial tissue (Davies et al., 1998; Landen et al., 2008). E-cadherin is uniformly expressed in ovarian inclusion cysts, benign ovarian cancers and ovarian cancer (Imai et al., 2004; Hudson et al., 2008). We found that E-cadherin was significantly upregulated after treatment with 5μM DZNep for 48 h.

In summary, this report contributes to the growing understanding of the role of DZNep in the treatment of ovarian cancers. We demonstrated that DZNep had the ability to inhibit proliferation and induce apoptosis in A2780 in vitro. We also demonstrated that DZNep suppresses migration of A2780. Further studies are required to more fully elucidate the mechanisms of the effects of DZNep on A2780.

Acknowledgements

The author(s) declare that they have no competing interests.

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