

Vascular endothelial growth factor modulates cisplatin sensitivity in human ovarian carcinoma cells

Research Article

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Abbreviations: VEGF, vascular endothelial growth factor; VPF, vascular permeability factor; GSH, glutathione; cisplatin (CDDP), cis-diamminedichloroplatinum (II); bFGF, basic fibroblast growth factor; HGF, hepatocyte growth factor; PGF, placenta growth factor; PDEGF, platelet-derived endothelial growth factor; PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5 diphenyl tetrazolium bromide; RT-PCR, reverse transcriptase polymerase chain reaction; FasL, Fas ligand; NER, nucleotide excision repair; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/ERK kinase; AP-1, activator protein 1.

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Summary

Cisplatin is among the most effective and widely used chemotherapeutic agents employed for treatment of human cancers, and a major limitation of cisplatin chemotherapy is serious drug resistance. Vascular endothelial growth factor (VEGF), a potent angiogenic factor, plays an important role in cell growth and survival of endothelial cells and tumor cells. However, the role of VEGF in cisplatin resistance in human cancers is unclear. Therefore, the present study sought to examine the effect of VEGF on cisplatin-induced cytotoxicity in human ovarian cancer CaOV3 cells. We show in this report that VEGF mediated cytoprotection against cisplatin-caused cell killing and significantly increased cell survival in CaOV3 cells exposed to cisplatin. VEGF was found to reduce cisplatin cytotoxicity and decrease cisplatin sensitivity in these cells, which are dependent upon the concentrations of cisplatin. The effect of VEGF was also sequence-dependent. Concurrent treatment of VEGF and cisplatin markedly increased cell viability as compared to cells exposed to cisplatin alone. By contrast, only a little effect of VEGF was observed when cells were treated with VEGF after or prior to cisplatin. These findings suggest that VEGF may contribute to the chemoresistance to cisplatin in patients with ovarian cancer and other tumors, and hence highlight that potential therapeutic strategies of anti-angiogenesis which specifically inhibit VEGF activity may reverse drug resistance to cisplatin.

I. Introduction

Human ovarian cancer is the fifth leading cause of cancer death among women in the United States and the most common cause of death in women in whom gynecologic cancer develops. The mainstay of therapy for advanced stage ovarian cancer is cisplatin-based systemic chemotherapy (Young et al, 1993; Reed, 1993; Reed, 1996; Reed et al, 1996; Reed, 1998). However, long-term disease-free survival following appropriate aggressive initial treatment ranges from 10 to 20% (Young et al, 1993; Omura et al, 1991). The disappointing survival

statistics stem from the fact that while most patients have a response to initial therapy, the majority of these responses are transient. Most patients will have cisplatin-resistant disease. The precise mechanism of cisplatin resistance in human cancers is, however, still not fully understood although substantial efforts have been made to solve this enigma. Multiple mechanisms have been implicated in the development of cisplatin resistance including reduced accumulation of the drug, elevated levels of glutathione (GSH), enhanced expression of metallothionein, increased DNA repair, enhanced tolerance of cisplatin damage, increased levels of Bcl-2-related anti-apoptosis genes, and

alterations in signal transduction pathways involved in apoptosis (Reed et al, 1996; Gosland et al, 1996; Dabholkar and Reed, 1996; Kerbel, 1997; Reed, 1998; Reed, 1998).

Angiogenesis is the process of new blood vessel growth and is necessary for growth of solid malignant tumors (Folkman, 1991). Angiogenesis not only allows a tumor to increase in size, but also increases the probability of metastasis (Folkman, 1993). Vessel growth is controlled by a balance of endogenous inhibitors and stimulators (Folkman, 1991). A number of growth factors and cytokines have been identified as potential positive inducers of angiogenesis, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), placenta growth factor (PGF), and platelet-derived endothelial growth factor (PDEGF) (Kerbel, 2000; Slodkowska et al, 2000; Liekens et al, 2001). Recently, an increasing number of studies both *in vitro* and in mice demonstrated that angiogenic growth factors augment tumor cell survival and confer drug resistance by inhibiting apoptosis (Borsellino et al, 1995; Volm et al, 1999; Grothey et al, 1999; Coleman et al, 2000). For instance, evidence showed that HGF reduces sensitivity to chemotherapeutic agents and stimulates cell invasion and migration (Meng et al, 2000). Other investigations indicated that elevated levels of intracellular bFGF correlate with resistance to fludarabine in chronic lymphocytic leukemia (Menzel et al, 1996). Furthermore, overexpression of bFGF is associated with resistance to cisplatin in a human bladder cancer cell line (Miyake et al, 1998). Moreover, the addition of exogenous bFGF to endothelial cells inhibits apoptosis induced by DNA damage from ionizing radiation (Fuks et al, 1994). However, the role of VEGF and other angiogenic factors in the development of cisplatin drug resistance is unknown at the present time. The goal of the current study was to evaluate the effect of VEGF on cisplatin antitumor activity in human ovarian cancer cells. We show in this paper that VEGF decreases drug sensitivity and increases cell survival in human CaOV3 ovarian tumor cells exposed to cisplatin.

II. Materials and methods

A. Cell line and cell culture conditions

The human ovarian carcinoma cell line CaOV3 (HTB-75; American Type Culture Collection, Manassas, VA) that has been described previously was used in all of the experiments. Cells were cultured in monolayers using a RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamine, 0.2 units/ml human insulin, 50 units/ml penicillin, and 50 µg/ml streptomycin (Life Technologies, Inc, Gaithersburg, MD). Cells were grown in logarithmic growth at 37 °C in a humidified atmosphere consisting of 5% CO₂ and 95% air. Cells were routinely tested for mycoplasma infection using a commercial assay system (MytoTect; Life Technologies); new cultures were established monthly from frozen stocks. All media and reagents contained <0.1 ng/ml endotoxin as determined by Limulus polyphemus amebocyte lysate assay (Whittaker Bioproducts, Walkersville, MD). Cell viability was determined in triplicate by trypan blue dye exclusion. Before starting the experiments, cells were grown to 70-90% confluence after

subculturing. Cisplatin (Sigma-Aldrich Co., St. Louis, MO) was initially dissolved in phosphate-buffered saline without Ca²⁺ or Mg²⁺ at 1.0 mg/ml (3.33 µM cisplatin), and dilutions from this solution were made in medium to obtain the desired drug treatment concentrations. VEGF (Human Recombinant VEGF165) was purchased from Oncogene Research Products (Cambridge, MA). VEGF was initially dissolved in phosphate-buffered saline (PBS) at 5 µg/ml, and dilutions from this solution were made in medium to obtain the desired cytokine treatment concentrations.

CaOV3 cells were assayed for sensitivity to cisplatin by measurement of the inhibition of growth following 24 to 48-h exposure to cisplatin ranging from 20 to 40 µM. Cells were seeded at an initial cell density of 2 X 10⁴ cells/ml. Cells were starved for 48 h with the medium containing 0.2% fetal bovine serum. Cells were then treated with VEGF or cisplatin alone, or the combination of VEGF and cisplatin in different sequences. After continuous contact with cisplatin for 24-48 h, medium was removed, and cell viabilities were determined using the MTT cell viability assay. Cells treated similarly in the absence of VEGF and/or cisplatin served as controls.

B. Cell toxicity assay

The effect of VEGF and/or cisplatin on antitumor activity in human CaOV3 ovarian carcinoma cells was determined by the MTT survival assay, or using a commercial MTT assay kit (CellTiter 96 Aqueous One Solution Cell Proliferation Assay; Promega Corporation, Madison, WI) according to the manufacturer's instructions. The MTT survival assay was performed as described previously (Yu et al, 2000). The MTT assay is a commonly used method in evaluation of cell survival, based on the ability of viable cells to convert MTT, a soluble tetrazolium salt [3-(4,5-dimethylthiazole-2-yl)-2,5 diphenyl tetrazolium bromide], into an insoluble formazan precipitate, which is quantitated by spectrophotometry following solubilization in dimethyl sulfoxide (DMSO). Briefly, CaOV3 cells untreated and treated with VEGF or cisplatin alone, or the combination of VEGF and cisplatin in 96-well tissue culture dishes were incubated with MTT (2 µg/ml) for 4 h. The cells were then solubilized in 125 µl of DMSO and absorbance readings were taken using a 96-well Optsys MR Microplate Reader (ThermoLabsystems; Chantilly, VA). The amount of MTT dye reduction was calculated based on the difference between absorbance at 570 nm and at 630 nm. Cell viability in treated cells was expressed as the amount of dye reduction relative to that of untreated control cells. The wells which contained only medium and 10 µl of MTT were used as blanks for the plate reader. Three sets of experiments were performed in 8-12 wells for each treatment.

III. Results

A major goal of the ongoing project is to understand whether angiogenic growth factors that induce angiogenesis might reverse the drug resistance to cisplatin in human ovarian cancer and better understand the underlying mechanisms in the process. In the present investigation, we first determined whether the angiogenic factor VEGF could influence the cisplatin anticancer activity in human ovarian carcinoma cells. VEGF was found to significantly reduce cell susceptibility to cell killing caused by cisplatin and augment cell survival in the CaOV3 human ovarian tumor cell line. As shown in **Fig. 1**, concurrent treatment of both VEGF and cisplatin for 24

h dramatically decreased cisplatin-induced cell killing in these cells from 80% cells to 32-47% cells. This amounts to approximately a 2.5-fold reduction in the amount of cell killing as compared to the control in which cells were treated with cisplatin alone. A greater effect of VEGF was observed in cells exposed to VEGF plus cisplatin for 24 h, and then fresh medium containing only VEGF was replenished for an additional 24 h. In contrast, only a little effect of VEGF in this regard was seen when VEGF was given after or prior to cisplatin.

We also examined the cytoprotective effect of VEGF in cells exposed to 20 μM cisplatin for a longer time (48 h) and in cells exposed to a higher concentration of cisplatin at 40 μM . In each case, the effect of VEGF was virtually identical to the effect seen in cells exposed to 20 μM cisplatin for 24 h. **Figure 2** shows that there was marked cell kill in the cisplatin-treated group, in which approximately 87% of the cells were killed in a 48-h incubation time. By contrast, VEGF treatment significantly diminished the cell kill in this model system, yielding a 4.7 to 5.8-fold higher level of cell viability than in cells exposed to cisplatin alone.

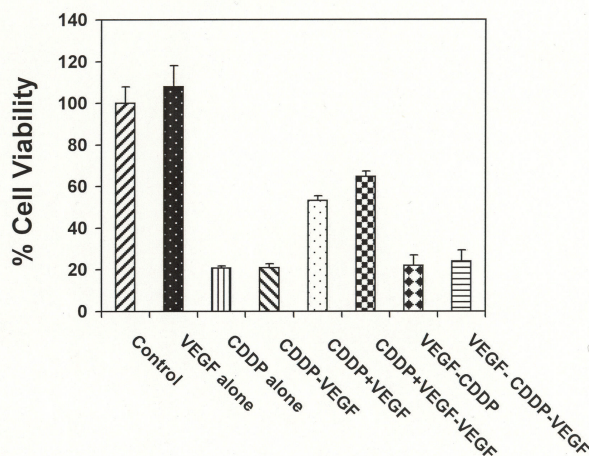


Figure 1. Effect of VEGF on cytotoxicity by CDDP (20 μM for 24 h) in human ovarian carcinoma cells as assessed by the MTT survival assay.

2×10^4 cells per well from CaOV3 cells were evenly distributed in 96-well plates, and were starved for 48 h in culture medium containing 0.2% fetal bovine serum. Cells were then treated as the following: Control, treated with medium only; VEGF alone, treated with 50 ng/ml VEGF only; CDDP alone, exposed to CDDP at 20 μM for 24 h, and fresh medium was then replenished; CDDP-VEGF, exposed to CDDP for 24 h, and then fresh medium containing VEGF was replenished; CDDP+VEGF, exposed to CDDP and VEGF for 24 h, and then fresh medium was replenished; CDDP+VEGF-VEGF, exposed to CDDP and VEGF for 24 h, and then fresh medium containing VEGF was replenished; VEGF-CDDP, treated with VEGF for 24 h, changed to fresh medium containing CDDP for another 24 h, and then fresh medium was replenished; VEGF-CDDP-VEGF, treated with VEGF for 24 h, changed to fresh medium containing CDDP for an additional 24 h, and then fresh medium containing VEGF was replenished. All the cells were harvested 48 h from the time when 20 μM CDDP was added to the culture. Cell viability was measured by the MTT assay and is expressed as a percentage of untreated control. CDDP, cisplatin.

The same was true when CaOV3 cells were exposed to 40 μM of cisplatin.

Our data in **Fig. 3** shows that VEGF, at the concentration of 50 ng/ml, both decreased cisplatin-induced cytotoxicity and increased cell survival in these cells. **Table I** is the comparison of the effect of VEGF on cell viability between different concentrations of cisplatin, or different exposure time to the drug in human CaOV3 ovarian cancer cells.

As seen in the table, there is no significant difference in the effect of VEGF on cell toxicity between the cells exposed to cisplatin for 24 h or for 48 h. However, the cell viability following cisplatin and VEGF treatment was much lower in cells exposed to 40 μM cisplatin than in cells exposed to 20 μM cisplatin, indicating that the higher the concentration of cisplatin, the lower the protective effect of VEGF. Together, these results suggest that VEGF has strong cytoprotective activity against cisplatin-caused cell death and promotes cell survival in cisplatin-treated human CaOV3 ovarian cancer cells.

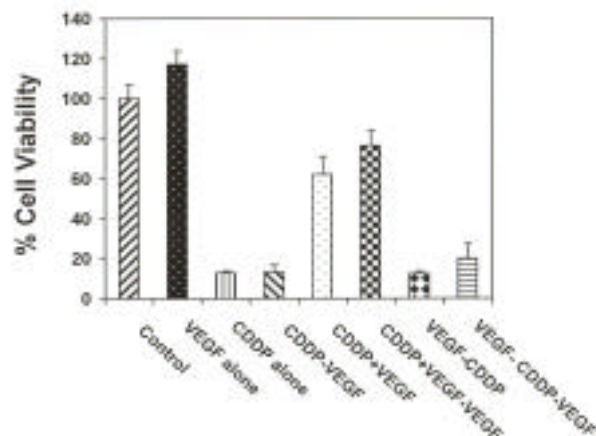


Figure 2. Effect of VEGF on cytotoxicity by CDDP (20 μM for 48 h) in human ovarian cancer cells as determined by the MTT cell viability assay.

2×10^4 cells per well from CaOV3 cells were evenly plated in 96-well plates, and were starved for 48 h in culture medium containing 0.2% fetal bovine serum. Cells were then treated as the following: Control, treated with medium only; VEGF alone, treated with 50 ng/ml VEGF only; CDDP alone, exposed to CDDP at 20 μM for 48 h, and fresh medium was then replenished; CDDP-VEGF, exposed to CDDP for 48 h, and then fresh medium containing VEGF was replenished; CDDP+VEGF, exposed to CDDP and VEGF for 48 h, and then fresh medium was replenished; CDDP+VEGF-VEGF, exposed to CDDP and VEGF for 48 h, and then fresh medium containing VEGF was replenished; VEGF-CDDP, treated with VEGF for 24 h, changed to fresh medium containing CDDP for 48 h, and then fresh medium was replenished; VEGF-CDDP-VEGF, treated with VEGF for 24 h, changed to fresh medium containing CDDP for 48 h, and then fresh medium containing VEGF was replenished. All the cells were harvested 72 h from the time when 20 μM CDDP was added to the culture. Cell viability was measured by the MTT assay and is expressed as a percentage of untreated control. CDDP, cisplatin.

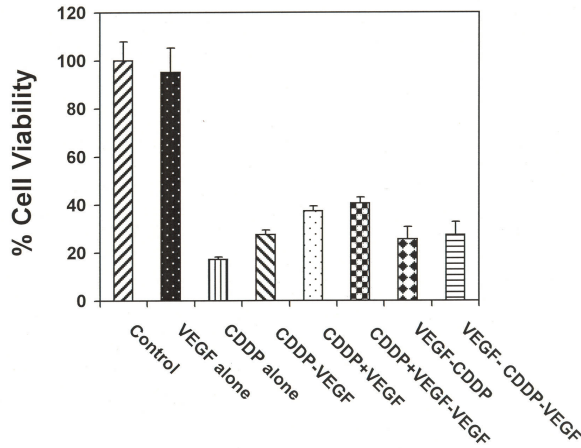


Figure 3. Effect of VEGF on cell toxicity by CDDP (40 μ M for 24 h) in human ovarian tumor cells as measured by the MTT survival assay.

2 X 10⁴ cells per well from CaOV3 cells were evenly distributed in 96-well plates, and were starved for 48 h in culture medium containing 0.2% fetal bovine serum. Cells were then treated as the following: Control, treated with medium only; VEGF alone, treated with 50 ng/ml VEGF only; CDDP alone, exposed to CDDP at 40 μ M for 24 h, and fresh medium was then replenished; CDDP-VEGF, exposed to CDDP for 24 h, and then fresh medium containing VEGF was replenished; CDDP+VEGF, exposed to CDDP and VEGF for 24 h, and then fresh medium was replenished; CDDP+VEGF-VEGF, exposed to CDDP and VEGF for 24 h, and then fresh medium containing VEGF was replenished; VEGF-CDDP, treated with VEGF for 24 h, changed to fresh medium containing CDDP for an additional 24 h, and then fresh medium was replenished; VEGF-CDDP-VEGF, treated with VEGF for 24 h, changed to fresh medium containing CDDP for another 24 h, and then fresh medium containing VEGF was replenished. All the cells were harvested 48 h from the time when 40 μ M CDDP was added to the culture. Cell viability was determined by the MTT assay and is expressed as a percentage of untreated control. CDDP, cisplatin

IV. Discussion

VEGF, also known as vascular permeability factor (VPF), is a cytokine/growth factor, and has been known to be a potent, endothelial-cell-specific angiogenic mitogen. VEGF is secreted from tumor cells and other cells via its specific binding to its tyrosine kinase receptors (VEGFR1/Flt-1 and VEGFR2/Flk-1/KDR). Binding of VEGF to its receptors leads to intracellular propagation of a mitogenic signal through activation of the PI3 kinase-Akt and the *ras-raf*-MAP kinase pathways. VEGF and its receptors are expressed in angiogenic tissues during development, wound healing, and other situations such as neoplasm (Boocock et al, 1995) when angiogenesis occurs. Evidence has been accumulated that VEGF and its receptor mRNAs or proteins have been identified by reverse transcriptase polymerase chain reaction (RT-PCR), *in situ* hybridization, or immunohistochemistry in a number of tumors, including ovarian cancer (Boocock et al, 1995). The spatial and temporal patterns of expression of VEGF and its receptors as well as the results of targeted mutagenesis support that they are required for both normal and pathological angiogenesis during development. Similarly, the role of VEGF in tumor angiogenesis has been clearly demonstrated using tumor models in rodents. Moreover, recent studies also found that VEGF plays a role in the regulation of apoptosis induction and cell survival. Thus, VEGF contributes to the development and progression of malignant tumors.

However, the role of VEGF and its receptor tyrosine kinases in the formation and development of drug resistance in human cancers remains unknown. In the present study, we demonstrated for the first time that VEGF plays an important role in the modulation of cisplatin antitumor activity in human ovarian carcinoma cells.

Table I. Comparison of the effect of VEGF on cell viability between CDDP at different concentrations or exposure time in human CaOV3 ovarian cancer cells.

Treatment ^a	CDDP (dose and time)		
	20 μ M, 24 h ^b	30 μ M, 48 h ^b	40 μ M, 24 h ^b
Control	100	100	100
VEGF alone	107	117	96
CDDP alone	20	13	17
CDDP-VEGF	21	14	27
CDDP+VEGF	53	62	37
CDDP+VEGF-VEGF	68	76	41
VEGF-CDDP	23	13	26
VEGF-CDDP-VEGF	24	20	28

^aSee Figures 1-3 legends for additional details.

^bCell viabilities were determined by the MTT survival assay and expressed as a percentage of untreated control. CDDP, cisplatin

The addition of exogenous VEGF to the growth medium of CaOV3 cells markedly enhanced the dose-dependent survival of cells exposed to increasing concentrations of cisplatin, and therefore directly reduced the sensitivity of CaOV3 cells to this chemotherapy drug. The cytoprotective effect of VEGF against cisplatin toxicity is sequence-dependent, with maximal effect seen in cells exposed to VEGF and cisplatin simultaneously, suggesting that VEGF may exert its action through reducing cisplatin-caused cell damage. A higher survival rate was observed in cells treated with VEGF plus cisplatin for 24 h, followed by VEGF only for an additional 24 h, as compared to the cells incubated with medium only after VEGF and cisplatin were removed from the cultures. This appears to suggest that continuous exposure of the cells to VEGF after cisplatin damage may prevent the cells from further damage or apoptosis caused by cisplatin, or it may enhance cell repair of cisplatin-induced DNA damage leading to a higher rate of cell viability. Exogenous VEGF did not produce any cytotoxic effects in the absence of cisplatin, and it had the expected stimulatory effect on cell growth (Figures 1 and 2). Similar effect of VEGF was also observed in TOV-21G human ovarian cancer cell line, indicating that augmented cell survival and decreased cisplatin sensitivity appear to be a common effect of VEGF in different ovarian cancer cells.

The mechanism underlying the effect of VEGF in ovarian tumor cells is, however, unclear at this point. Given its broad spectrum of activities, VEGF may exert its effect in mediating the development of drug resistance through several ways. First of all, VEGF may be involved in cisplatin drug resistance via anti-apoptotic activity. Recently, experimental and clinical studies showed that VEGF was related not only to angiogenic activity, but also to the inhibition of apoptotic activity (Słodkowska et al, 2000). For example, the effects of VEGF on delaying apoptosis and prolonging the survival of tumor cell may be indirect, via the inhibition of specific genes that promote apoptosis, such as down-regulating Fas and Fas ligand (FasL) proteins, or decreasing levels of cytochrome c in the cytoplasm (Volm et al, 1996; Coleman et al, 2000). Alternatively, VEGF may block cisplatin-induced apoptosis through reducing cisplatin-caused DNA damage. Cisplatin-induced apoptosis has been closely tied to its ability to cause DNA damage (Eastman, 1990).

In addition, VEGF-mediated protection of tumor cells against cisplatin may result not only from activation of an anti-apoptosis pathway, but also from an increase in repair of DNA damage. In other words, VEGF may modulate cisplatin sensitivity indirectly through the regulation of DNA repair activity. Although we do not have direct evidence at this point that VEGF mediate this effect by enhancing DNA repair, we showed in a separate study that SU5416, a selective inhibitor of VEGF receptors, counteracted the effect of VEGF by augmenting cisplatin cytotoxicity and increasing cisplatin sensitivity in human ovarian tumor cells. We further found that the effect of SU5416 on the increase in cell death or reduction of cell survival of cisplatin-treated cells is due in part to the reduction in repair efficiency of cisplatin-caused DNA

damages. It is broadly accepted that the antitumor activity of cisplatin results from the formation of cisplatin-DNA adducts that strongly interfere with the processing of genomic information (Rosenberg, 1979; Reed et al, 1993; Dabholkar and Reed, 1996). Cisplatin-DNA damage is repaired predominantly by the nucleotide excision repair (NER) machinery. Enhanced DNA repair capacity contributes to the formation of drug resistance to cisplatin in a wide variety of tumor cells.

Our and other previous studies revealed that cisplatin may increase NER repair gene expression and DNA repair activity through a JNK-AP1 pathway leading to cell survival (Potapova et al, 1997; Li et al, 1998; Li et al, 1998; Li et al, 1999). On the other hand, a great deal of studies have supported the general view that activation of the ERK pathway delivers a survival signal. Consistent with such a prosurvival function for ERK, studies have shown that an inhibition of ERK signaling leads to increased sensitivity of ovarian cancer cell lines to cisplatin (Hayakawa et al, 1999; Persons et al, 1999). Therefore, it is possible that JNK and ERK may act collaboratively or synergistically to enhance survival of cisplatin-treated cells, as inhibition of either pathway accentuated cisplatin toxicity (Hayakawa et al, 1999). Based on these observations, we propose that VEGF may stimulate a *ras-raf*-MEK-ERK or PI3K-Akt cascade activity that enhances cisplatin-induced activation of JNK-AP1. Such a mechanism might serve to integrate the actions of receptor protein tyrosine kinases and non-receptor protein tyrosine kinases, which may underlie the mechanism of VEGF and cisplatin mediated DNA repair and cell survival in human ovarian cancer and other carcinomas. Studies are in progress to explore whether VEGF mediates cytoprotection against cisplatin-induced apoptosis in human cancer cells by upregulating apoptosis-rescue signals, assess the effect of VEGF on DNA repair activity, and elucidate the role of PI3K-Akt, ERK or JNK in the signal transduction pathways through which VEGF modulates DNA repair activity or apoptotic activity in human carcinoma cells.

In conclusion, we provided *in vitro* evidence for the first time that VEGF mediated cytoprotection against cisplatin-caused cell killing and significantly increased cell survival in human ovarian cancer cells exposed to cisplatin. Taken together with previous studies, our results strengthen the case that VEGF contributes to the carcinogenesis and chemoresistance of the chemotherapeutic agent cisplatin. Strategies targeting VEGF signaling pathway or the activity of VEGF, or down-regulating its expression could be employed to reduce drug resistance, increase tumor cell apoptosis, and enhance the chemotherapeutic effectiveness of cisplatin.

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References

- Boocock CA, Sharkey AM, McLaren J, Barker PJ, Wright KA, Twentyman PR, Smith SK (1995) Expression of vascular endothelial growth factor and its receptors flt and KDR in ovarian carcinoma. **J. Natl. Cancer Inst.** 87, 506-516.
- Borsellino N, Belledgrun A, Bonavida B (1995) Endogenous interleukin 6 is a resistance factor for cis-diaminedichloroplatinum and etoposide-mediated cytotoxicity of human prostate carcinoma cell lines. **Cancer Res.** 55, 4633-4639.
- Coleman AB, Momand J, Kane SE (2000) Basic fibroblast growth factor sensitizes NIH 3T3 cells to apoptosis induced by cisplatin. **Molecular Pharmacology** 57, 324-333.
- Dabholkar M, Reed E (1996) Cisplatin. **Cancer Chemother. Biol. Response Modif.** 16, 88-110.
- Eastman A (1990) Activation of programmed cell death by anticancer agents: cisplatin as a model system. **Cancer Cells** 2, 275-280.
- Folkman J (1971) Tumor angiogenesis: Therapeutic implications. **N. Engl. J. Med.** 285, 1182-1186.
- Folkman J (1993) Diagnostic and therapeutic applications of angiogenesis research. **C. R. Acad. Sci. III** 316, 909-918.
- Fuks Z, Persaud RS, Haimovitz-Friedman A (1994) Basic fibroblast growth factor protects endothelial cells against radiation-induced programmed cell death *in vitro* and *in vivo*. **Cancer Res.** 54, 2582-2590.
- Gosland M, Lum B, Schimmelpfennig J, Baker J, Doukas M (1996) Insights into mechanisms of cisplatin resistance and potential for its clinical reversal. **Pharmacotherapy** 16, 16-39.
- Grothey A, Voigt W, Schmoll HJ (1999) The role of insulin-like growth factor I and its receptor in cell growth, transformation, apoptosis, and chemoresistance in solid tumors. **J. Cancer Res. Clin. Oncol.** 125, 166-173.
- Hayakawa J, Ohmichi M, Kurachi H, Mercola D, Murata Y (1999) Inhibition of extracellular signal-regulated protein kinase or c-Jun N-terminal protein kinase cascade, differentially activated by cisplatin, sensitizes human ovarian cancer cell line. **J. Biol. Chem.** 274, 31648-31654.
- Kerbel RS (1997) A cancer therapy resistant to resistance. **Nature** 390, 335-336.
- Kerbel RS (2000) Tumor angiogenesis: past, present and the near future. **Carcinogenesis** 21, 505-515.
- Li Q, Ding L, Yu JJ, Mu C, Tsang B, Bostick-Bruton F, Reed E (1998) Cisplatin and phorbol ester independently induce ERCC-1 protein in human ovarian tumor cells. **Int. J. Oncol.** 13, 987-992.
- Li Q, Gardner K, Zhang L, Tsang B, Bostick-Bruton F, Reed E (1998) Cisplatin induction of ERCC-1 mRNA expression in A2780/CP70 human ovarian cancer cells. **J. Biol. Chem.** 273, 23419-23425.
- Li Q, Tsang B, Bostick-Bruton F, Reed E (1999) Modulation of ERCC-1 messenger RNA expression by pharmacological agents in human ovarian carcinoma cells. **Biochem. Pharmacol.** 57, 347-353.
- Liekens S, Clercq ED, Neyts J (2001) Angiogenesis: regulators and clinical applications. **Biochem. Pharmacol.** 61, 253-270.
- Meng Q, Rosen EM, Fan S (2000) Hepatocyte growth factor decreases sensitivity to chemotherapeutic agents and stimulates cell adhesion, invasion, and migration. **Biochem. Biophys. Res. Commun.** 274, 772-779.
- Menzel T, Rahman Z, Gabilove J (1996) Elevated intracellular level of basic fibroblast growth factor correlates with stage of chronic lymphocytic leukemia and is associated with resistance to fludarabine. **Blood** 87, 1056-1063.
- Miyake H, Hara I, Kamidono S (1998) Expression of basic fibroblast growth factor is associated with resistance to cisplatin in a human bladder cancer cell line. **Cancer Lett.** 123, 121-126.
- Omura GA, Brady MF, Park RC (1991) Long-term follow-up and prognostic factor analysis in advanced ovarian carcinoma: The Gynecologic Oncology Group experience. **J. Clin. Oncol.** 9, 1138-1150.
- Persons DL, Cui W, Pelling JC (1999) Cisplatin-induced activation of mitogen-activated protein kinases in ovarian carcinoma cells: inhibition of extracellular signal-regulated kinase activity increases sensitivity to cisplatin. **Clin. Cancer Res.** 5, 1007-1014.
- Potapova O, Haghghi A, Bost F, Liu C, Birrer MJ, Gjerset R, Mercola D (1997) The Jun kinase/stress-activated protein kinase pathway functions to regulate DNA repair and inhibition of the pathway sensitizes tumor cells to cisplatin. **J. Biol. Chem.** 272, 14041-14044.
- Reed E (1993) Platinum analogs, anticancer drugs. In *Cancer Principles and Practice of Oncology* Rosenberg SA, Ed. Philadelphia, PA, Lippincott, pp. 390-400.
- Reed E (1996) The chemotherapy of ovarian cancer. **PPO Updates** 10, 1-12.
- Reed E (1998) Nucleotide excision repair and anti-cancer chemotherapy. **Cytotechnology** 27, 187-201.
- Reed E (1998) Platinum-DNA adduct, nucleotide excision repair and platinum based anti-cancer chemotherapy. **Cancer Treatment Reviews** 24, 331-344.
- Reed E, Dabholkar M, Chabner BA (1996) Platinum analogues. In *Cancer Chemotherapy and Biotherapy: Principles and Practice*, 2nd ed. Longo DL, Ed. Philadelphia, PA, Lippincott-Raven Publishers, pp. 357-378.
- Reed E, Parker RJ, Gill I, Bicher A, Dabholkar M, Vionnet JA (1993) Platinum-DNA adduct in leukocyte DNA of a cohort of 49 patients with 24 different types of malignancies. **Cancer Res.** 53, 3694-3699.
- Rosenberg B (1979) Anticancer activity of cis-dichlorodiammineplatinum (II) and some relevant chemistry. **Cancer Treat. Rep.** 63, 1433-1438.
- Slodkowska J, Sikora J, Roszkowski-Sliz K (2000) Expression of vascular endothelial growth factor and basic fibroblast growth factor receptors in lung cancer. **Analyt. Quant. Cytol. Histol.** 22, 398-402.
- Snedecor GW, Cochran WG (1967) *Statistical methods*. Ames, IA, The Iowa State University Press.
- Volm M, Koomagi R, Mattern J (1996) Interrelationships between microvessel density, expression of VEGF and resistance to doxorubicin of non-small lung cell carcinoma. **Anticancer Res.** 16, 213-218.
- Volm M, Mattern J, Koomagi R (1999) Inverse correlation between apoptotic (Fas ligand, Caspase-3) and angiogenic factors (VEGF, Microvessel density) in squamous cell lung carcinomas. **Anticancer Res.** 19, 1669-1672.
- Young RC, Perez CA, Hoskins WJ (1993) *Cancer of the ovary*. In *Cancer-Principles & Practice of Oncology*, 4th ed. Rosenberg SA, Ed. Philadelphia, PA, J. B. Lippincott, pp. 1245-1252.
- Yu JJ, Li Q, Reed E (2000) Comparison of two human ovarian carcinoma cell lines (A2780/CP70 and MCAS) that are

equally resistant to platinum, but differ at codon 118 of the ERCC1 gene. **Int. J. Oncol.** 16, 555-560.



Front row from left: Qingdi Q. Li and Sean Ryan
Rear row from left: Hang Hu, Guodong Hu, Xiping Li and Gangduo Wang

