

Overexpression of angiogenic growth factors in lung cancer cells is associated with cisplatin resistance

Research Article

Xiping Li^{1, 2}, Xuyi Liu³, Jie Wang³, Zengli Wang², Wei Jiang³, Eddie Reed¹, Yi Zhang⁴, Yuanlin Liu⁴, and Q. Quentin Li^{1*}

¹Mary Babb Randolph Cancer Center and Department of Microbiology, Immunology and Cell Biology, West Virginia University School of Medicine and Robert C. Byrd Health Sciences Center, Morgantown, WV 26506, USA; ²Department of Respiratory Medicine, Sichuan University Huaxi Medical Center, Chengdu 610041, P. R. China; ³Department of Medicine, Peking University School of Oncology, Beijing 100036, P. R. China; ⁴Institute for Basic Medical Research, Chinese Academy of Military Medical Sciences, Beijing 100850, P. R. China

*Correspondence: Q. Quentin Li, M.D., Ph.D., 1831 Mary Babb Randolph Cancer Center, West Virginia University Health Sciences Center, P.O. Box 9300, Morgantown, WV 26506-9300, USA, Tel: 304-293-6870; Fax: 304-293-4667; E-mail: qli@hsc.wvu.edu

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Summary

Cisplatin is among the most effective agents in the treatment of human lung cancer, and the development of resistance to this drug is the main reason that results in chemotherapy failure in the clinic. Recent evidence showed that angiogenesis growth factors, including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), augment tumor cell growth and survival, and confer drug resistance by inhibition of apoptosis. However, the relationship between angiogenesis and drug resistance in human cancer remains poorly understood. We therefore conducted this study to investigate the expression of angiogenic growth factors and drug-resistance related genes in cisplatin-sensitive and cisplatin-resistant human lung cancer cells. We report in this work that the levels of the mRNA and protein expression of VEGF and bFGF were strikingly elevated in resistant A₅₄₉^{DDP} lung cancer cells than those in parental A₅₄₉ lung cancer cells. We also found that the levels of multidrug resistance-related protein (MRP) and c-erbB-2 were significantly higher in A₅₄₉^{DDP} resistant cells when compared with A₅₄₉ parental cells. As expected, lung resistance protein (LRP) was expressed only in A₅₄₉^{DDP} resistant cells but not in A₅₄₉ parental cells. Interestingly, there was a strong correlation between bFGF and c-erbB-2 or bFGF and MRP in these cells. These findings indicate that the overexpression of VEGF and bFGF, as well as the drug-resistance related genes, is associated positively with cisplatin resistance in human lung cancer cells, and therefore support the potential therapeutic applications of anti-angiogenics in regulating cisplatin sensitivity in resistant lung cancer and other tumors.

I. Introduction

Lung cancer is the major cause of death from all human malignancies in the United States. *cis*-Diamminedichloroplatinum (II) (cisplatin, DDP) is one of the most effective drugs currently available for treatment for a wide variety of solid tumors, including lung cancer, bladder cancer, ovarian cancer, testicular cancer, and head and neck cancer (Reed, 1993; Reed, 1996; Reed et al, 1996). One of the hurdles with cisplatin treatment is the clinical development of resistance to this drug (Dabholkar and Reed, 1996; Gosland et al, 1996; Reed et al, 1996; Reed, 1998; Reed, 1998). Multiple mechanisms have been implicated in the development of cisplatin resistance, including reduced cisplatin uptake or decreased accumulation of the drug, elevated levels of glutathione

and metallothionines, 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 (Dabholkar and Reed, 1996; Gosland et al, 1996; Reed et al, 1996; Reed, 1998; Reed, 1998). However, the mechanism by which cells develop resistance to cisplatin is far from clear at this time. Therefore, intense research is needed to solve this problem because it is a major impediment to the clinical success of the drug.

Angiogenesis, the formation of new blood vessels, is essential for normal reproduction, development, and organ repair. Angiogenesis is also important in a variety of tumor processes, such as tumor growth and metastasis and drug resistance (Kerbel, 2000; Stavrovskaya, 2000;

Liekens et al, 2001). These processes are regulated differentially by a variety of distinct pro-angiogenic molecules and anti-angiogenic molecules (Slodkowska et al, 2000). The angiogenic switch is mediated by the balance of angiogenic inducers and angiogenic inhibitors. A number of growth factors, cytokines, chemokines, enzymes, and adhesion molecules have been identified as potential positive regulators of angiogenesis so far. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are among the most important ones (Kerbel, 2000; Liekens, 2001).

Recently, experimental and clinical studies are accumulating to show that angiogenic factors augment tumor cell growth and survival, and confer drug resistance by inhibition of apoptotic activity (Volm et al, 1999; Coleman et al, 2000). However, the relationship between angiogenesis and cisplatin drug resistance in human tumors is poorly understood. The present investigation was therefore designed to study the expression of the angiogenesis growth factors VEGF and bFGF in cisplatin-sensitive and cisplatin-resistant human lung cancer cell lines. We also set out to examine the expression of the drug-resistance related genes lung resistance protein (LRP), multidrug resistance-related protein (MRP), c-erbB-2 and bcl-2, and analyze the relationships between the angiogenic factors and the drug-resistance related genes in these model systems. We report herein the results of this investigation.

II. Materials and methods

A. Cell lines and cell culture conditions

The human lung adenocarcinoma parental cell line A549 and the cisplatin-resistant cell line A549^{DDP} were cultured in DMEM medium containing 10 % fetal calf serum, at 37 °C in a humidified 5% CO₂ incubator.

Both cell lines were assayed for sensitivity to cisplatin by measurement of the inhibition of growth following 48-h exposure to cisplatin ranging from 0.1 to 1,000 μM. Cell lines were seeded at an initial cell density of 5×10^4 cells/ml. After continuous contact with the drug for 48 h, medium was removed, and cell viabilities were determined by using MTT assay. Cells treated similarly in the absence of drug served as controls.

B. RNA isolation and RT-PCR analysis

Total RNA was extracted from cell lines using Trizol reagent (GIBCO-BRL, Gaithersburg, MD, USA), according to the standard acid-guanidium-phenol-chloroform method. The RT-PCR was performed using the TaKaRa RNA RT-PCR Kit (TaKaRa Shuzo Co., Ltd., CA, USA). The reverse-transcribed total volume was 20 μl per sample. It included that MgCl₂ 4 μl, 10X RNA PCR buffer 2 μl, RNase free H₂O 7.5 μl, dNTP 2 μl, RNase inhibitor 0.5 μl, reverse transcriptase 1 μl, random primer 1 μl, RNA 2 μl. Placed all tubes in a thermal cycler and set the parameters by the following conditions: 30 °C 10 min, 46 °C 30 min, 99 °C 5 min, and 5 °C 5 min per cycle. The reverse transcriptase reaction product was served as a template DNA for PCR amplification. -actin cDNA was used as an internal reference. Total volume of PCR was 50 μl per sample. It included that 10 μRNA PCR buffer 5 μl, 25 mM MgCl₂ 3 μl, 10 μM dNTP 1 μl, 10 μM primer: sense 1 μl and antisense 1 μl,

TagTM 0.5 μl, cDNA 2 μl, sterilized distilled water 36.5 μl. The amplification for VEGF was done for 5 min at 95 °C, 1 min at 94 °C, 1.5 min (last 2 cycles, 2 min) at 58 °C, and 2 min (last 2 cycles, 5 min) at 72 °C for a total of 32 cycles. For bFGF was 40 sec (last 3 cycles, 1.5 min) at 94 °C, 1.3 min (last 3 cycles, 2 min) at 48 °C, and 1.2 min (last 3 cycles, 2 min) at 72 °C for a total of 28 cycles. For MRP and LRP: 94 °C 4 min; 94 °C 30 sec, 55 °C 1 min, and 72 °C 2 min for a total of 30 cycles. For bcl-2 and c-erbB-2: 94 °C 4 min; 94 °C 30 sec, 55 °C 30 sec, and 72 °C 30 sec for a total of 30 cycles. All genes were extended thoroughly at 72 °C for 10 min. The PCR primers for the target gene cDNA were listed in **Table 1**. Electrophoresis was performed with 10 μl of PCR products in 1.8% agarose gel. The electrophoretogram was scanned. The relative mRNA expression levels of target genes were calculated with the optical density (OD) values from the target genes and -actin.

C. Immunocytochemistry

The protein expression of VEGF, bFGF, MRP, LRP, bcl-2, and c-erbB-2 in A549 and A549^{DDP} cells was assessed by immunocytochemistry using an anti-VEGF monoclonal antibody (1:100) and an anti-bFGF polyclonal antibody (1:200) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), as well as anti-MRP, LRP, bcl-2 and c-erbB-2 monoclonal antibodies (1:20-1:40) (Beijing Zhongshan Biotech Company, Beijing, China). The samples were stained by SP staining. All reagents were used in the negative controls except the primary antibodies.

D. Statistical analysis of data

Data were analyzed for significance using the student's *t* test. The relationship between the data was analyzed statistically by *Pearson* correlation test. The criterion for statistical significance was $p < 0.05$.

III. Results

A. The cytotoxicity of cisplatin to cisplatin-sensitive and cisplatin-resistant human lung adenocarcinoma cell lines

Sensitivity to cisplatin was determined by measuring inhibition of cell growth following continuous exposure of cells to concentrations of the drug ranging from 0.1 to 1,000 μM cisplatin for 48 h. The A549 human lung adenocarcinoma parental cell line and A549^{DDP} cisplatin-resistant cell line exhibited cisplatin IC₅₀ values of 1 μM and 10 μM, respectively (**Figure 1**).

B. The mRNA expression of angiogenic growth factors and drug-resistance related genes in cisplatin-sensitive and cisplatin-resistant human lung cancer cells

Reverse transcription PCRs were performed to analyze the levels of mRNA expression of the angiogenic factors and the drug-resistance related genes. **Table 1** shows the primers used and the PCR-amplified products for each gene in our experiments. **Figure 2** presents the relative mRNA expression of the angiogenesis growth factors and the drug-resistance related genes in both cisplatin resistant A549^{DDP} lung cancer cells and A549 parental cells.

Table 1. RT-PCR primers and amplification products of VEGF, bFGF, MRP, LRP, bcl-2, c-erbB-2 and β -actin genes.

Gene	Primer	Amplification product
VEGF	Sense: 5'-GAA GTG GTG AAG TTC ATG GAT GTC-3'	541 bp, 408 bp
	Antisense: 5'-CGA TCA TTC TGT ATC AGT CTT TCC-3'	
bFGF	Sense: 5'-GTG TGT GCT AAC CGT TAC CT-3'	237 bp
	Antisense: 5'-GCT CTT AGC AGA CAT TGG AAG-3'	
MRP	Sense: 5'-TCT CTC CCG ACA TGA CCG AGG-3'	140 bp
	Antisense: 5'-CCA GGA ATA TGC CCC GAC TTC-3'	
LRP	Sense: 5'-GTC TTC GGG CCT GAG CTG GTG TCG-3'	221 bp
	Antisense: 5'-CTT GGC CGT CTC TTG GGG GTC CTT-3'	
bcl-2	Sense: 5'-GTG GAG GAG CTC TTC AGG GA-3'	304 bp
	Antisense: 5'-AGG CAC CCA GGG TGA TGC AA-3'	
c-erbB-2	Sense: 5'-GAT GTA TTT GAT GGT GAC CT-3'	183 bp
	Antisense: 5'-ATC TGG CTG GTT CAC ATA TT-3'	
β -actin	Sense: 5'-ATC TTC AAA CCT CCA TGA TG-3'	120 bp
	Antisense: 5'-ACC CCC ACT GAA AAA GAT GA-3'	

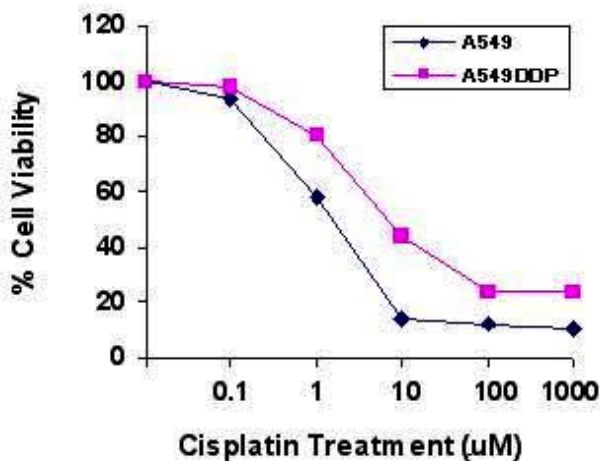


Figure 1. The sensitivity of A549 and A549^{DDP} human lung adenocarcinoma cell lines to cisplatin.

5 X 10⁴ cells per well from A549 cells or A549^{DDP} cells were evenly plated in 96-well plates. Cells were then exposed to cisplatin at 0.1, 1, 10, 100, or 1,000 µM for 48 h. Cell viability was measured by the MTT assay 48 h after drug exposure and is expressed as a percentage of the untreated control.

Compared to cisplatin-sensitive cells, cisplatin-resistant cells show consistently higher mRNA levels of VEGF, bFGF, MRP, LRP, and c-erbB-2. The relative mRNA levels of expression were 10.03 for VEGF, 3.08 for bFGF, 0.96 for MRP, 2.07 for c-erbB-2, and 0.81 for LRP. In A549 parental cells, the relative mRNA levels of expression for VEGF was 1.81, 1.53 for bFGF, 0.53 for MRP, 0.84 for c-erbB-2, and was not detectable for LRP. The differences between A549^{DDP} resistant cells and A549 parental cells in relative mRNA expression levels for VEGF, bFGF, MRP, c-erbB-2 and LRP were statistically significant (all $p < 0.01$) (Table 2).

C. The protein expression of angiogenic growth factors and drug-resistance related genes in cisplatin-sensitive and cisplatin-resistant human lung carcinoma cells

Our experimental results from immunocytochemistry showed that the levels of protein expression of VEGF, bFGF, and MRP were all higher in A549^{DDP} resistant cells, as compared with A549 parental cells. While LRP and c-erbB-2 were negative in A549 parental cells, they were positive in A549^{DDP} resistant cells (Table 2; Figures 3, 4, 5, 6, 7 and 8).

D. The relationship between angiogenic growth factors and drug-resistance related genes in A549 and A549^{DDP} human lung adenocarcinoma cells

Pearson correlation test has been used to measure the degree to which two genes may show concurrent increased or decreased mRNA expression levels. Comparison of

bFGF and MRP shows correlative expression of these two genes in A549 parental cells ($r = 0.979$; $p = 0.032$). A comparison of bFGF and c-erbB-2 shows that mRNA levels for bFGF were also correlated with c-erbB-2 in A549^{DDP} resistant cells ($r = 1$; $p = 0.004$). The relationships shown suggest that as bFGF mRNA increases, so do MRP and c-erbB-2 mRNAs

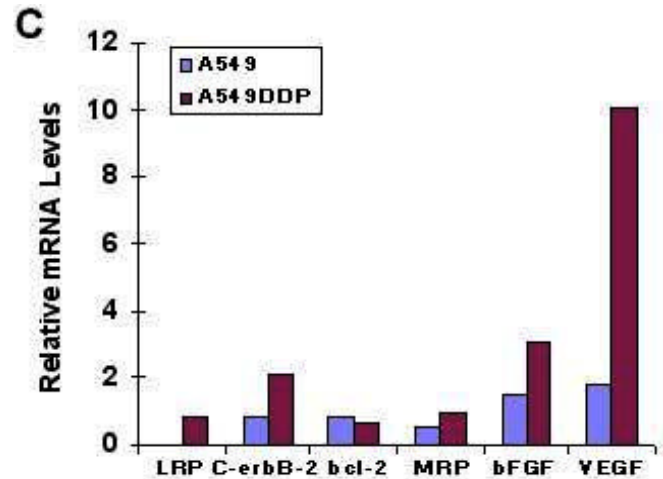
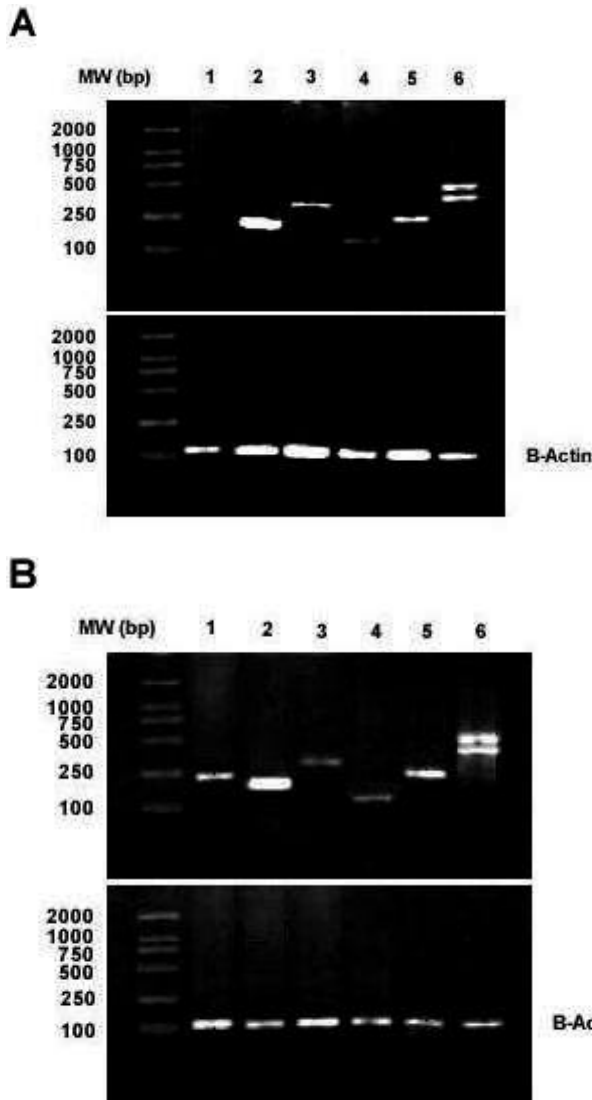


Figure 2. The levels of the mRNA expression of angiogenic growth factors and drug-resistance related genes in cisplatin-sensitive and cisplatin-resistant human lung adenocarcinoma cell lines.

RT-PCR analysis of the mRNA levels of VEGF, bFGF, MRP, LRP, c-erbB-2, and bcl-2 in cisplatin-sensitive human A549 lung adenocarcinoma cells (A) and in cisplatin-resistant human A549^{DDP} lung adenocarcinoma cells (B). A 541-bp and a 408-bp segments of VEGF cDNA, a 237-bp segment of bFGF cDNA, a 140-bp segment of MRP cDNA, a 221-bp segment of LRP cDNA, a 304-bp segment of bcl-2 cDNA, a 183-bp segment of c-erbB-2 cDNA, and a 120-bp segment of -actin cDNA were amplified by RT-PCR, respectively, and the aliquots of amplified DNAs were electrophoresed through a 1.8% agarose gel. The relative mRNA levels of the target genes were quantified by densitometry and expressed as a ratio to -actin, and these values are shown graphically in panel (C). 1, LRP; 2, c-erbB-2; 3, bcl-2; 4, MRP; 5, bFGF; 6, VEGF.

IV. Discussion

Although significant progress has been made in the treatment of lung cancer with combination chemotherapy, lung cancer remains the leading cause of cancer death (American Cancer Society, 2000). Platinum-containing anti-tumor drugs are the most commonly used agents for the treatment of lung carcinoma (Reed, 1993; Dabholkar and Reed, 1996; Reed et al, 1996). Toxicities and emergence of drug-resistant tumors, however, are major problems preventing curative therapy (Reed, 1993; Dabholkar and Reed, 1996; Reed et al, 1996). While several mechanisms of resistance to cisplatin have been identified (Gosland et al, 1996), no single mechanism can clearly explain cisplatin drug-resistance in lung cancer. Understanding the relationship between angiogenesis and

drug resistance and the molecular basis involved could lead to strategies resulting in improved therapeutic benefits to patients with cisplatin resistant carcinomas.

In our study, we found that the levels of the angiogenic growth factors VEGF and bFGF expression were dramatically increased in cisplatin resistant A549^{DDP} lung cancer cells as compared to A549 parental cells, suggesting a role of angiogenic growth factors in the formation of cisplatin drug resistance in these cells. We also confirmed the previous observations by others that LRP, MRP, bcl-2 and c-erb-2 are actively associated with drug resistance in lung cancer. However, the mechanism underlying the relationship between angiogenesis and drug resistance, as well as the role of angiogenic growth factors in the development of cisplatin drug resistance in human tumors remains unclear at this time.

Table 2. The comparison of the levels of mRNA and protein expression of angiogenic growth factors and drug-resistance related genes between cisplatin-sensitive and cisplatin-resistant human lung cancer cells.

Gene	A549		A549 ^{DDP}		P value ^c
	mRNA (X±SD) ^a	Protein ^b	mRNA (X±SD) ^a	Protein ^b	
VEGF	1.81 ± 0.03	++	10.03 ± 0.38	++++	p<0.01
bFGF	1.53 ± 0.04	++	3.08 ± 0.06	++++	p<0.01
MRP	0.53 ± 0.04	+	0.96 ± 0.02	++	p<0.01
bcl-2	0.84 ± 0.14	+++	0.71 ± 0.13	+++	p=0.731
e-erbB-2	0.84 ± 0.06	-	2.07 ± 0.09	++	p<0.01
LRP	0	-	0.81 ± 0.03	++	p<0.01

^aRT-PCR analysis of mRNA levels of VEGF, bFGF, MRP, bcl-2, e-erbB-2 and LRP in cisplatin-sensitive human A549 lung adenocarcinoma cells and in cisplatin-resistant human A549^{DDP} lung adenocarcinoma cells, as described under "Materials and methods". Values for each gene are means of three independent experiments. For each gene, the band optical density value was divided by its β-actin value (see Figure 2 for additional details).

^b-, negative; +, low level; ++, moderate level; +++, high level; +++, very high level of protein expression of the genes listed in the table, as determined by immunocytochemistry (see "Materials and methods" for additional details).

^cStatistical analysis for the comparison of the levels of mRNA expression of the genes between A549 cells and A549^{DDP} cells.

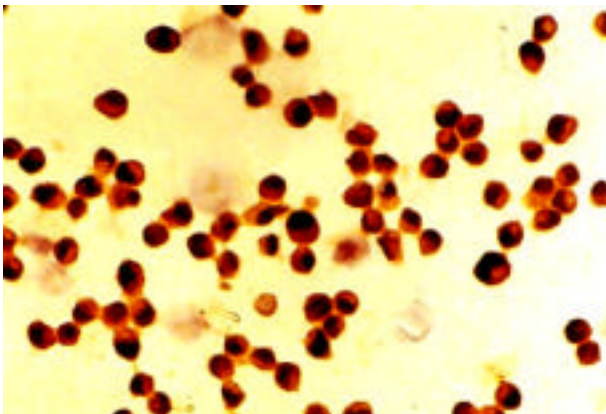


Figure 3. VEGF protein levels in cisplatin-resistant A549^{DDP} human lung cancer cells. Immunohistochemistry staining with an anti-VEGF monoclonal antibody (1:100) (Santa Cruz, CA, USA) shows that VEGF protein is strongly positive in human lung A549^{DDP} adenocarcinoma cells (SP staining × 40).

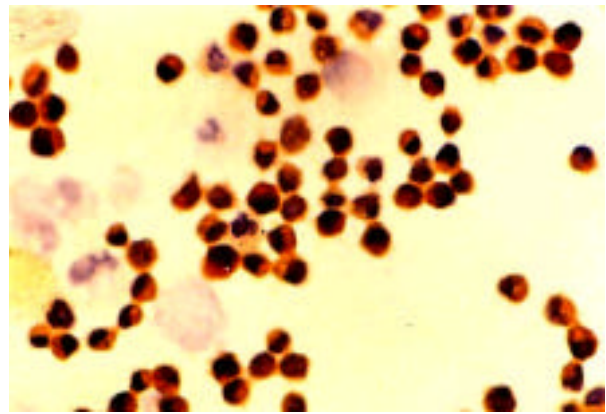


Figure 5. bcl-2 protein expression in cisplatin-resistant A549^{DDP} human lung cancer cells. Immunohistochemistry staining with an anti-bcl-2 monoclonal antibody shows that bcl-2 protein is strongly positive in human lung A549^{DDP} adenocarcinoma cells (SP staining × 40).

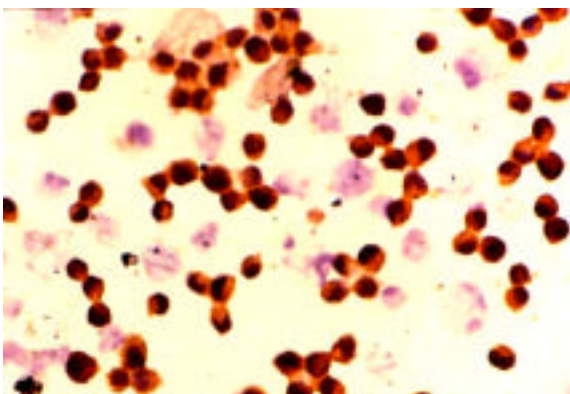


Figure 4. bFGF protein levels in cisplatin-resistant A549^{DDP} human lung cancer cells. Immunohistochemistry staining with an anti-bFGF polyclonal antibody (1:200) (Santa Cruz, CA, USA) shows that bFGF protein is strongly positive in human lung A549^{DDP} adenocarcinoma cells (SP staining × 40).

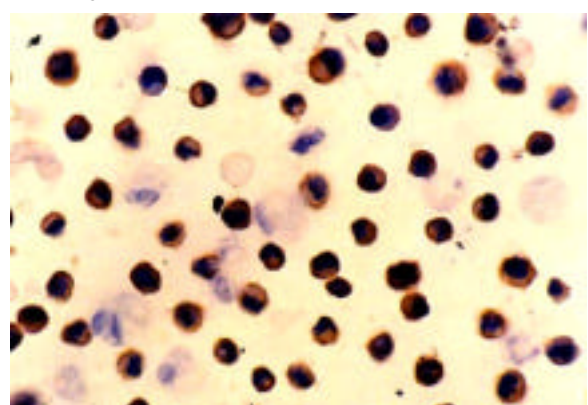


Figure 6. MRP protein expression in cisplatin-resistant A549^{DDP} human lung cancer cells. Immunohistochemistry staining with an anti-MRP monoclonal antibody shows that MRP protein is positive in human lung A549^{DDP} adenocarcinoma cells (SP staining × 40).

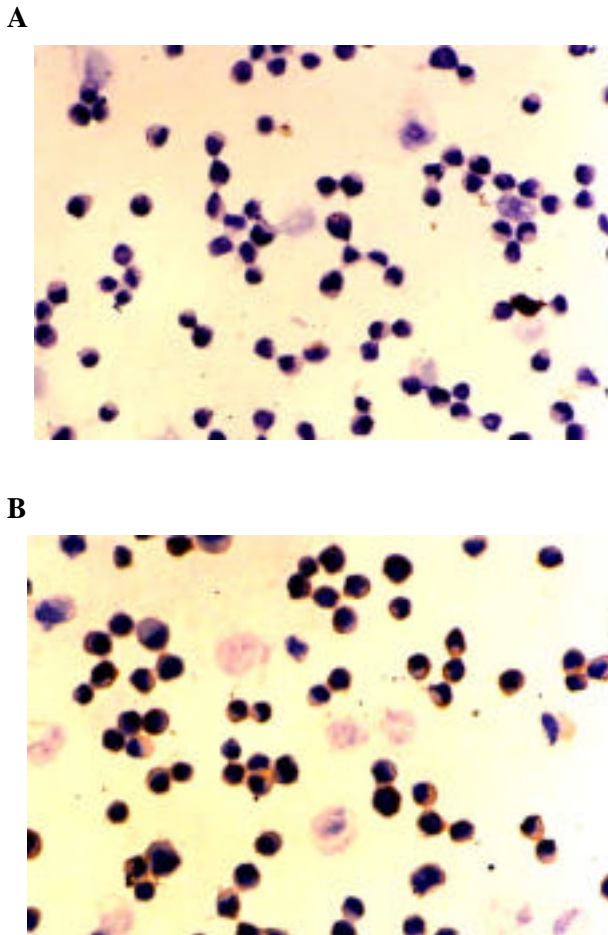


Figure 7. The levels of c-erbB-2 protein expression in cisplatin-sensitive A549 and cisplatin-resistant A549^{DDP} human lung cancer cells. Immunohistochemistry staining with an anti-c-erbB-2 monoclonal antibody shows that c-erbB-2 protein is negative in the human lung adenocarcinoma cell line A549 (A), but it is positive in human lung A549^{DDP} adenocarcinoma cells (B) (SP staining _40).

The MRP gene is a member of the ATP-binding cascade (ABC) transporter superfamily. It is involved in the efflux of cytotoxic drugs. MRP overexpression can lead to reduced drug access to its intracellular target, by increasing drug efflux and/or by altering its intracellular distribution (Kuwano et al, 1999). LRP is the main human vault protein. It mediated the drug resistance to cisplatin and alkylating agents by becoming involved in the rapid drug distribution from the nucleus to cytoplasmic vesicles. LRP can reduce the drug concentration in the nucleus and decrease the drug effect on DNA targets (Martin et al, 1998). The c-erbB-2 gene belongs to the epidermal growth factor receptor family. It is involved in the regulation of a variety of vital functions controlled by any of the erbB-receptor family members, including cell growth, differentiation, and apoptosis.

IV. Discussion

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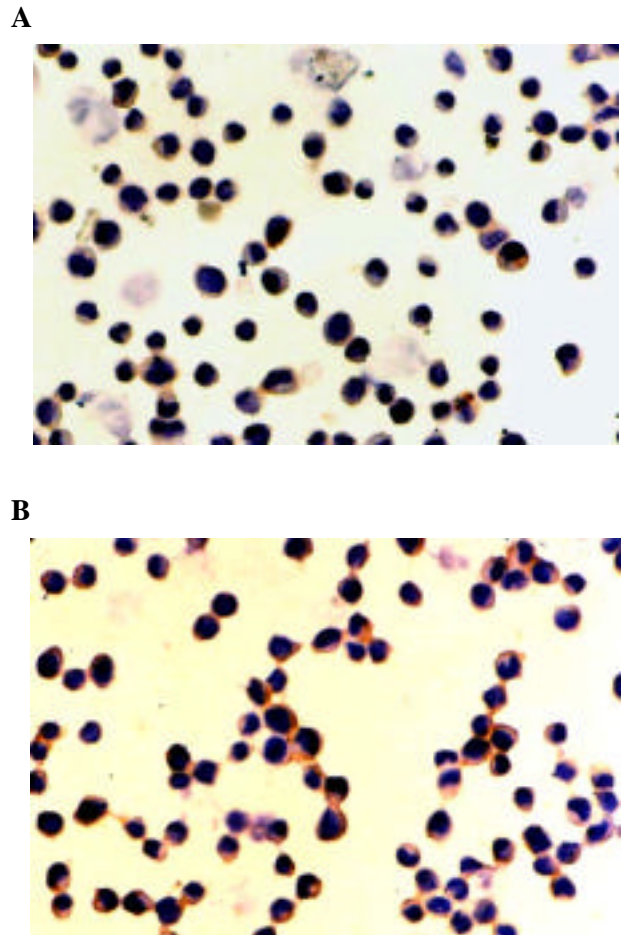


Figure 8. The levels of LRP protein expression in cisplatin-sensitive A549 and cisplatin-resistant A549^{DDP} human lung cancer cells. Immunohistochemistry staining with an anti-LRP monoclonal antibody shows that LRP protein is negative in the human lung adenocarcinoma cell line A549 (A), but it is positive in human lung A549^{DDP} adenocarcinoma cells (B) (SP staining _40).

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The MRP gene is a member of the ATP-binding cascade (ABC) transporter superfamily. It is involved in the efflux of cytotoxic drugs. MRP overexpression can lead to reduced drug access to its intracellular target, by increasing drug efflux and/or by altering its intracellular distribution (Kuwano et al, 1999). LRP is the main human vault protein. It mediated the drug resistance to cisplatin and alkylating agents by becoming involved in the rapid drug distribution from the nucleus to cytoplasmic vesicles. LRP can reduce the drug concentration in the nucleus and decrease the drug effect on DNA targets (Martin et al, 1998). The c-erbB-2 gene belongs to the epidermal growth factor receptor family. It is involved in the regulation of a variety of vital functions controlled by any of the erbB-receptor family members, including cell growth, differentiation, and apoptosis. Data from laboratory studies showed that higher levels of p185c-erbB-2 expression in tumor cell lines were correlated with increased resistance to Taxol (Yu et al, 1998)). Our study showed that the expression of MRP, LRP and c-erbB-2 in cisplatin drug-resistant cells was significantly higher than that in parental cells. These results suggest that these genes may be involved in the formation and development of cisplatin drug resistance in A549^{DDP} lung cancer cells. Although the precise mechanism involved is unclear now, it has been demonstrated that the overexpression of MRP confers resistance to the chemotherapy-induced apoptosis that is associated with the overexpression of anti-apoptotic genes and the downregulation of pro-apoptotic genes (Gupta et al, 1998). Further studies are necessary to investigate the mechanisms that may be responsible for the role of these genes in mediating the formation of the cisplatin resistance phenotype in lung cancer and other tumors.

Angiogenesis is important in a variety of processes, such as growth, metastasis and resistance (Stavrovskaya, 2000, Liekens et al, 2001). The angiogenic cascade is regulated differentially by a variety of distinct pro-angiogenic molecules, as well as a number of anti-angiogenic molecules (Slodkowska et al, 2000). VEGF, endothelial cell-specific mitogen and angiogenesis factor, is emerging as a major regulator of normal and pathologic angiogenesis (Volm et al, 1999; Volm et al, 1999). bFGF is a multifunctional molecule that belongs to a family of fibroblast growth factors. It appears to play a role in embryonic development, tumor invasion, wound healing, and angiogenesis (Gupta et al, 1998). VEGF and bFGF may be involved in tumorigenesis via proliferative and anti-apoptotic activities. Recently, experimental and clinical studies showed that VEGF and bFGF were related not only to angiogenic activity, but also to rapid tumor growth and the inhibition of apoptotic activity (Slodkowska et al, 2000).

Our study showed that although VEGF and bFGF were all expressed in A549 and A549^{DDP} cells, the levels of

the angiogenic growth factor expression in A549^{DDP} were strikingly higher than those in A549 cells, indicating an association between angiogenic growth factor expression and cisplatin drug resistance in the lung carcinoma cells. Little is known at present about the exact role of VEGF and bFGF in the process and the underlying mechanisms. Given their broad spectrum of activities, angiogenic growth factors may play an important role in mediating the development of drug resistance through several ways. Firstly, angiogenic growth factors can lead to drug resistance by suppression of tumor cell apoptosis. For example, the effects of VEGF and bFGF on delaying apoptosis and prolonging the survival of tumor cell may be indirect, via the induction of one or more of cytokines or inhibition of specific genes that promote apoptosis (Volm et al, 1996; Coleman et al, 2000). Secondly, angiogenic growth factors may modulate cisplatin sensitivity indirectly through the regulation of the activity of MRP, c-erbB-2, or some other drug-resistance related genes in tumor cells. As an indication, we show in this work that bFGF expression is correlated positively with c-erbB-2 and MRP expression in these cells. Thirdly, tumor vasculature is often inadequate for the tumor mass because the rate of neovascularization frequently fails to keep pace with tumor growth. A number of studies have indicated that VEGF may play an important role in tumor angiogenesis, and VEGF has been demonstrated to be upregulated by hypoxia. It has been shown that hypoxia can induce resistance to a number of antineoplastic agents. Hypoxia can also enhance genetic instability in tumor cells, which can lead to more rapid development of drug resistant tumor cells (Volm et al, 1996). Finally, we demonstrated recently in a separate study that VEGF mediated cytoprotection against cisplatin cell death and increased cell survival in cisplatin-resistant human tumor cells. Although the underlying mechanism is not known at this point, we showed that SU5416, a selective inhibitor of VEGF receptors, counteracted the effect of VEGF by enhancing cisplatin cytotoxicity and increasing cisplatin sensitivity in the 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. Enhanced DNA repair capacity contributes to the formation of drug resistance to cisplatin in a wide variety of tumor cells. Studies are under way to investigate whether angiogenic growth factors mediate protection against cisplatin-induced apoptosis in human cancer cells by upregulating apoptosis-rescue signals, assess the effect of angiogenic growth factors on DNA repair activity, and elucidate the role of PI3-kinase or Akt in the signal transduction pathway through which VEGF regulates DNA repair activity in human carcinoma cells.

In summary, we show in this article that the levels of the angiogenesis growth factors, VEGF and bFGF, and the drug-resistance related genes LRP, MRP and c-erbB-2 are significantly elevated in cisplatin-resistant human A549^{DDP} lung carcinoma cells, as compared to A549 parental cells. There is a strong correlation between the expression of the angiogenic growth factors and the drug-resistance related genes in the lung carcinoma cells. These data suggest that

the overexpression of VEGF and bFGF is strongly associated with cisplatin drug resistance in these lung cancer model systems. Therefore, our studies provide the rationale for the development of novel and potentially useful therapeutic strategies of anti-angiogenesis for cisplatin resistant malignancies.

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