Cisplatin Reduces Endothelial Cell Migration Via Regulation of Type 2-Matrix Metalloproteinase Activity

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Key Words

Endothelial cells • Cisplatin • Cell migration • Cell viability • Matrix metalloproteinases • Mitogen activated protein kinases

Abstract

Aims: In this study we investigated the effect of cisplatin on endothelial cell migration, an essential process for vascular remodeling and regeneration in several physiological and pathological situations. **Material and Methods:** Human umbilical vein endothelial cells (HUVEC) were treated with cisplatin and endothelial cell migration analyzed by fluorescence and scratch-wound migration assay. MMP2 and MMP9 activity were determined by zymographic assay, and MAPK activation by Western blotting analysis. **Results:** We demonstrated that cisplatin provoked a time- and dose-dependent decrease of HUVEC migration; this effect was clearly independent from its well known cytotoxic activity. In addition, cisplatin markedly reduced MMP2 activity in both conditioned media and cell lysates, increased p38 MAPK and JNK phosphorylation, but did not affect ERK phosphorylation. Endothelial cell migration was attenuated by treatment of cells with GM6001, a non-specific inhibitor of MMPs, or by a selective anti-MMP2

antibody. However, treatment of cells with SB202190 or SP600125, inhibitors of p38 MAPK and JNK respectively, did not affect HUVEC migration. **Conclusion:** These results suggested that cisplatin induced a reduction of endothelial cell migration through an inhibition of MMP2 activity by downstream signal transduction pathways independent of JNK and p38 MAPK activation.

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Introduction

Cell migration is a necessary process in various physiological and pathological conditions such as embryonic development, tissue remodeling and regeneration, wound healing, angiogenesis, tumor invasion and metastasis [1-3].

A growing body of evidence indicates that endothelial cell migration can be tightly regulated at the level of the different sequential events integrating this complex dynamic process [2], and that some family members of the mitogen activated protein kinases (MAPK) could participate as main intermediates of signal transduction pathways mediating endothelial cell migration [4]. Activation of extracellular signal-regulated kinase (ERK),

c-Jun N-terminal kinase (JNK) and p38 MAPK cascade in response to external stimuli activate intracellular signaling molecules and/or induce crucial alterations in several cytoskeleton-related proteins, including actin, focal adhesion kinase (FAK), paxillin, Jun, microtubule-associated proteins (MAP) and Spir that are essential for cell migration process [5-7].

Recently, it has been established that MAPK family members can also mediate some of the cell functions regulating matrix-degrading metalloproteinase (MMPs) activity and gene expression through activation of AP-1 and ETS transcription factors [8, 9]. MMPs, a family of zinc- and calcium-dependent peptidases capable of degrading a wide variety of extracellular matrix (ECM) components, play an important role in physiological tissue remodeling, as well as during pathological remodeling in diseases associated with an unbalanced turnover of their extracellular matrix, such as wound healing and tumor growth [10, 11]. In particular, MMP2 and MMP9, the two predominately expressed MMPs in endothelial cells capable of degrading native collagen type IV, have been demonstrated to be directly involved in endothelial cell migration and vascular remodeling during angiogenesis [12, 13], and in tumor cell growth and progression during metastasis [14].

Cisplatin (cis-diammine-dichloroplatinum) has been considered to be one of the most effective chemotherapeutic agents utilized for treatment of a wide variety of human solid tumors, and one of the most potent inducers of apoptosis and growth arrest in many cell types [15-17]. It is generally accepted that cytotoxicity of cisplatin is mediated through induction of apoptosis and arrest of the cell cycle resulting from its interaction with DNA, such as the formation of cisplatin-DNA adducts, which activate multiple signaling pathways [16]. Nevertheless, the effect and molecular mechanisms of cisplatin on other cellular functions remain to be understood. In this study we analyzed the role of cisplatin on endothelial cell migration. Our data showed that cisplatin markedly reduces human umbilical vascular endothelial cell (HUVEC) migration and MMP2 activity through signal transduction pathways independent of MAPK family member activation.

Material and Methods

Materials

Endothelial cell growth supplemented (ECGS), foetal bovine serum (FBS), heparin, M199 medium, bovine serum

albumin (BSA), antibiotics, protease inhibitors, calcein-AM, gelatin, cisplatin and other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). SB202190 and SP600125 were from Calbiochem-Novabiochem (San Diego, CA, USA). Monoclonal phospho-ERK antibody and polyclonal ERK, MMP2, MMP9 and clusterin- β antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA), while polyclonal phospho-p38 MAPK, p38 MAPK, phospho-JNK, JNK and caspase-3 antibodies were from Cell Signaling Technology (Beverly, MA, USA). Calibrated pre-stained standards, IgG-horseradish peroxidase conjugated and the chemiluminescence kits were obtained from Pierce (Rockford, IL, USA). Poly(vinylidene difluoride) membranes were from Millipore (Bedford, MA, USA) and Hyperfilm ECL was from Amersham (Buckinghamshire, UK). FALCON HTS fluoroblok inserts were from Becton Dickinson (Bedford, MA, USA).

Endothelial cell culture conditions

HUVEC were isolated as described by Jaffe et al. [18], seeded on a gelatin-coated flask and maintained in M199 medium supplemented with 20% FBS, 30 μ g/ml ECGS and 100 μ g/ml heparin, containing gentamicin (50 μ g/ml) and amphotericin B (0.25 μ g/ml). The cells were maintained in a water-saturated atmosphere of 5% CO₂ and 95% air at 37°C. Before the experiments, cells were harvested with 0.05% trypsin-EDTA solution and plated onto plastic 100 mm culture dishes until confluence.

Endothelial cell migration assay

Endothelial cell migration assay was carried out using 24-well fluorescence-opaque membrane inserts as we have previously described [19]. Briefly, HUVEC grown to confluence in complete M199 medium were labeled in situ with 5 μ g/ml calcein-AM for 2 h at 37°C. The monolayer was trypsinized and the cells suspended in M199 medium supplemented with 0.1% BSA were added to 8 μ m FALCON HTS fluoroblok insert at a density of 5×10^5 cells/insert. Cisplatin was added to upper chamber and medium containing 10% FBS to the lower chamber as a chemoattractant. After incubation at 37°C real time kinetics of cell migration was determined by taking readings at different times. Fluorescence of cells that had migrated through membrane inserts was measured on the Fluorescence Microplate Reader (BIO-TEK instruments, Winooski, VT, USA) using an excitation and emission wavelength of 485 and 530 nm, respectively. In addition, HUVEC migratory activity was assessed using a scratch-wound migration assay, where confluent monolayers in 6-well plates were scratched with a sterile plastic pipette tip giving rise to one acellular 1 mm-wide lane per well. After washing, cells were supplied with 1.5 ml complete medium in the absence or presence of cisplatin at the indicated concentrations. Wounded areas were photographed at zero time and after 3 and 6 h of incubation. The migrated distance of cells was calculated by measuring the distance from the wound edge to the maximally migrated cell in distinct border zones. The percentage of closure was calculated as migratory distance from both sides versus the distance lesser of the wound.

Fig. 1. Time-dependent effect of cisplatin on HUVEC migration. Cells in the upper half of the chamber, incubated in the absence (●) or the presence of 80 µM cisplatin (○), were allowed to migrate through a porous membrane towards the lower chamber containing 10%-serum medium as chemoattractant. Data from three experiments are expressed as a percentage of untreated cells at the beginning of experiments (time 0). *P<0.05 vs. their respective controls in the absence of treatment.

LDH assay

Medium collected from control and cisplatin treated cells were tested for lactate dehydrogenase (LDH) activity (Cytotoxicity Detection kit LDH, Roche, Mannheim, Germany) as a measure of necrotic cell death. The assays were conducted following the manufacturer's instructions in flat-bottomed wells of 96-well plates. Briefly, 100 µl of each sample was added to reaction reagent and incubated for 30 min at 25°C in the dark. Following incubation, the absorbance of samples was measured at 490 nm using an ELISA plate reader (Asys Hitech, Eugendorf, Austria).

Gelatin zymographic assay

After HUVEC treatment with cisplatin for the indicated time periods, medium was removed and cells were rinsed with ice-cold PBS and then lysed in 50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 1% Triton X-100, 1% deoxycholate, 0.2 mM Na_3VO_4 , 1 mM EGTA, 0.4 mM EDTA, 0.01% NaN_3 , 1 µg/ml of aprotinin and leupeptin, and 0.1 µg/ml of phenylmethylsulphonyl fluoride. Conditioned media and cell lysates were centrifuged at 10,000 g for 10 min at 4°C to remove insoluble material. Equal amounts of protein were separated under non-reducing conditions using gelatin-10% polyacrylamide zymography gels. Gels were incubated in 50 mM Tris-HCl, pH 7.4, containing 2.5% Triton X-100, for 60 min at room temperature, and then in 50 mM Tris-HCl, pH 7.4, containing 5 mM CaCl_2 , 0.2 M NaCl, 1% Triton X-100, for 16 hours at 37°C. The developing buffer was discarded and gels were stained with 0.1% Coomassie blue solution (10% methanol, 5% acetic acid) for 2 h. The gels were then de-stained with 5% acetic acid and 10% methanol until sharp bands were visualized.

Fig. 2. Dose-dependent effect of cisplatin on HUVEC migration. Confluent monolayers were wounded and a wound assay was performed as described in Material and Methods. Fresh culture medium was added, either in the absence or presence of cisplatin (Cis). Photographs were taken at the beginning of the assay and at the indicated time. Data from three experiments are expressed as a percentage of the values obtained with their respective controls at the beginning of experiments (time 0). *P<0.05. Results shown are from a representative experiment.

Preparation of endothelial cell extracts and Western blot analysis

HUVEC were incubated at 37°C with vehicle or agent as indicated. Cells were rinsed with ice-cold phosphate-buffered saline (PBS) containing 0.5 mM Na_3VO_4 and then collected in lysis buffer (50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 1% Triton X-100, 1% deoxycholate, 0.2 mM Na_3VO_4 , 1 mM EGTA, 0.4 mM EDTA, 1 µg/ml of aprotinin and leupeptin, and 0.1 µg/ml of phenylmethylsulphonyl fluoride). Lysates were centrifuged at 10,000 g for 15 min at 4°C to remove insoluble material, and equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis and transferred electrophoretically to poly(vinylidene difluoride) membranes. Membranes were incubated for 1 h in blocking buffer (10 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 5% non-fat dry milk, and 0.1% Tween-20) and then incubated overnight at 4°C with specific antibodies. Membranes were washed twice with

Fig. 3. Effect of cisplatin on HUVEC death. Cells were treated with 80 μ M cisplatin for different times (1 to 6 h). (A) Conditioned media were used for LDH activity measurement. Data from three experiments are expressed as a percentage of untreated cells. (B) Cell lysates were analyzed by Western blotting using caspase 3 and clusterin- β antibodies. Results shown are from a representative experiment.

Fig. 4. Effect of cisplatin on MMPs activity of HUVEC. Cells were treated with 80 μ M cisplatin for different times (1 to 6 h), and then conditioned media (A) or cell lysates (B) were used for MMP2 (●) and MMP9 (○) activity measurement by zymographic analysis. Data from 3-5 experiments are expressed as a percentage of untreated cells. * P <0.05. Results shown are from a representative experiment.

blocking buffer and incubated for 45 min with IgG-horseradish peroxidase conjugated as a secondary antibody. Finally, membranes were washed twice with blocking buffer and twice with washing buffer (10 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, and 0.1% Tween-20), incubated with enhanced chemiluminescence detection reagents and exposed to Hyperfilm ECL. Approximate molecular masses were estimated using calibrated prestained standards. To ensure that there were similar amounts of protein in each sample the same membranes were stripped off, reprobed with polyclonal ERK, p38 MAPK or JNK antibodies and developed with anti-rabbit IgG-horseradish peroxidase conjugated secondary antibody by ECL.

Protein analysis

Protein contents were measured using the Coomassie blue binding method of Bradford [20].

Data analysis

Results were expressed as mean $\pm$ SEM. Data were compared by ANOVA followed by the Newman-Keuls multiple comparison test using SigmaStat, version 2.01 (Jandel Scientific).

Results

Effect of cisplatin on HUVEC migration

Endothelial cell migration plays an important role in many physiological processes and in the development diseases. In this study, we firstly investigated the effect of cisplatin on HUVEC migration. As shown in Fig. 1, a time-dependent decrease in cell migration was observed

Fig. 5. Effect of GM6001 and MMP2 or MMP9 antibodies on HUVEC migration. Confluent monolayers were wounded and a wound assay was performed as described in Material and Methods. Fresh culture medium was added, either in the absence or presence of 30 μ M GM6001 and MMP2 or MMP9 antibodies. Photographs were taken at the beginning of the assay and after 6 h incubation. Data from three experiments are expressed as a percentage of untreated cells. * $P < 0.05$. Results shown are from a representative experiment.

in HUVEC treated with cisplatin. In addition, the ability of cisplatin to reduce endothelial cell migration was also confirmed by using a scratch wound-healing mobility assay. As shown in Fig. 2, treatment of HUVEC with cisplatin reduced endothelial cell migration in a time- and dose-dependent manner. Taken together, these results indicated that cisplatin reduced endothelial cell mobility.

In order to rule out the likelihood that cisplatin also provoked cell death during the time period used for migration analysis we assayed LDH release, caspase-3 activation and clusterin- β expression. We found that the release of intracellular LDH in cisplatin-treated cells was unaltered (Fig. 3A), indicating that cisplatin does not cause cell membrane damage characteristic of necrosis. Moreover, no changes in caspase-3 cleavage or clusterin- β expression was found, thus suggesting that the decrease of endothelial cell migration provoked by cisplatin was not due to apoptosis (Fig. 3B).

Effect of cisplatin on MMP2 and MMP9 activities in HUVEC

Cell migration is a complex process that requires the coordinated contribution of numerous molecules,

Fig. 6. Effect of cisplatin on MAPK activity of HUVEC. Cells were treated with 80 μ M cisplatin for different times (1 to 6 h), and then cell lysates were analyzed by Western blotting using ERK1/2 (○), p38 MAPK (●) and JNK (■) antibodies. Results shown are from a representative experiment. Data from 3-5 experiments are expressed as a percentage of untreated cells.

including MMPs. To test whether cisplatin exerts any effect on MMP2 and MMP9 activity, the main MMPs expressed in endothelial cells, we evaluated the activities of both in conditioned media and cell lysates using zymographic analysis. Treatment of HUVEC with cisplatin caused a time-dependent reduction of MMP2 activity in conditioned media and cell lysates from HUVEC; conversely, the activity of MMP9 was undetectable in cell lysates and remained unchanged in conditioned media (Fig. 4).

Effect of MMPs on HUVEC migration

Acquisition of MMP activity is temporally associated with increased cell migration. To investigate whether the observed reduction of MMPs activity was involved in cisplatin-provoked HUVEC migration decrease, we used GM6001, a non specific inhibitor MMPs, and an antibody against MMP2 and MMP9 to inhibit HUVEC mobility. As shown in Fig. 5, treatment of cells with 30 μ M GM6001 or incubation with a specific antibody against MMP2, but not with anti-MMP9 antibody, markedly reduced HUVEC migration (Fig. 5), mimicking the inhibitory effect of cisplatin. These results suggest that MMP2 was involved

Fig. 7. Effect of p38 MAPK and JNK on HUVEC migration and MMP2 activity. (A) Confluent cells pretreated with 10 μ M SB202190 or 20 μ M SP600125 for 45 min were wounded and a wound assay in the presence or the absence of 80 μ M cisplatin was performed as described in Material and Methods. (B) MMP2 activity in conditioned media was analyzed by zymographic analysis. Data from three experiments are expressed as a percentage of untreated cells. * P <0.05. Results shown are from a representative experiment.

in the anti-migratory action of cisplatin on endothelial cells.

Effect of cisplatin on MAPK phosphorylation in HUVEC

It has been reported that activation of MAPK signaling plays a central role in the therapeutic activity of cisplatin. In the present study, Western blot analysis was used to determine whether cisplatin was able to induce ERK, p38 MAPK and JNK phosphorylation in endothelial cells. Our data show that cisplatin induced a time-dependent increase in p38 MAPK and JNK phosphorylation, without affecting the phosphorylation state of ERK (Fig. 6), thus suggesting that p38 MAPK and JNK could be downstream mediators of cisplatin action in endothelial cells.

Effect of MAPK on HUVEC migration

There is evidence that the different MAPK family members are involved in the regulation of cell migration. To test whether cell migration occurred subsequently to cisplatin-induced MAPK activation, we treated endothelial cells with inhibitors of p38 MAPK and JNK pathways. As shown in Fig. 7, both treatment of cells with 10 μ M SB202190, a specific p38 MAPK inhibitor, or with 20 μ M SP600125, a JNK inhibitor, decreased endothelial cell migration. However, pretreatment of HUVEC with these MAPK inhibitors had effect neither on cisplatin-induced anti-migratory action nor on MMP2 activity. Collectively, our data reveal that although p38 MAPK and JNK are two key mediators of endothelial

cell migration, the action of cisplatin on endothelial cell mobility occurs through downstream mechanisms not related with MAPK family member activation.

Discussion

Cisplatin is one of the most effective anti-tumor agents for the treatment of several carcinomas. Its mechanism of action has been primarily attributed to DNA binding and further activation of various intracellular signaling pathways that lead to induction of the apoptotic death of tumor cells [21]. In addition, cisplatin exerts other actions independent of DNA adduct production which have important consequences in the cell function. For example, cisplatin increases Cdk2 activity in mouse kidney proximal tubule cells which regulates G2-M phase cell-cycle progression [22] or promotes leukocytes adhesion to endothelial cells by stimulating ICAM-1 expression via a NF- κ B-dependent pathway [23]. However, little is known about the role of cisplatin in the regulation of endothelial cell migration, a critical cell event necessary for angiogenesis, tissue remodeling and wound healing, as well as for the growth and metastasis of many tumors [24].

In this study we clearly demonstrate the ability of this anti-tumor drug to cause a pronounced reduction of endothelial cell migration in vitro. Treatment of HUVEC with cisplatin induces a time- and dose-dependent decrease of cell migration, as determined by a continuous

fluorescence and a scratch-wound migration assay. Interestingly, the observed effect of cisplatin on endothelial cell migration was not associated with its well recognized cytotoxic action [21]. Treatment of endothelial cell with cisplatin did not cause cell membrane damage characteristic of necrosis, and was unable to activate caspase-3 or to induce the expression of apoptosis-related glycoprotein clusterin- β [25]. Altogether, our data suggests that cisplatin might have an indirect anti-neoplastic effect through the suppression of neovascularization required for tumor growth.

MMPs occupy a central role in several endothelial cell processes, such as cell motility, wound healing and angiogenesis, as well as during tumor invasion and/or metastasis. Previous reports have shown a variable effect of cisplatin on MMPs in different human cancer cells, suggesting a tumor-type modulation of these enzymes. Although an inhibitory action of cisplatin on MMP2 activity has been described in lung cancer and glioblastoma cells [26], other studies have shown that cisplatin does not exhibit a significant suppression of MMP2 activity in human cervical and ovarian carcinoma cells [27, 28]. To evaluate the effect of cisplatin on MMPs in vascular endothelial cells further studies were performed measuring MMP2 and MMP9 activity, the two most important MMPs expressed in endothelial cells, by zymographic analyses. Our finding reveals that MMP2 activity in conditioned media and cell lysates rapidly declined, in a time-dependent manner, following cisplatin treatment of endothelial cells, whereas MMP9 activity remained unchanged. Consistently with previous observations showing a differential regulation of MMP2 and MMP9 gene expression [29], our data suggest a possible MMP-type specific effect of cisplatin in endothelial cells.

A number of studies have reported that MMPs are directly implicated in the process of cell migration through degradation of extracellular matrix components [12, 13]. In an attempt to demonstrate the participation of MMPs as possible intermediary of cisplatin-induced endothelial cell migration reduction, further experiments were performed analyzing the effect of GM6001, a non-selective inhibitor of MMPs, and the specific anti-MMPs antibodies on HUVEC migration. The data presented here demonstrated that treatment of cells with GM6001 elicited a moderate reduction of the migratory capacity of HUVEC, showing the participation of MMPs in endothelial cell mobility. In addition, we used MMP2-blocking antibody to identify whether MMP2 is specifically an important mediator in this cell process.

According to previous findings anti-MMP2 antibodies, but not anti-MMP9 antibodies, notably attenuated endothelial cell mobility, suggesting that MMP2 was directly involved in the anti-migratory action of cisplatin on endothelial cells.

Cisplatin is able to activate MAPK pathways in various tumor cells and transformed cell lines through stimulation of different signaling proteins, such as protein kinase C (PKC), Ras superfamily members, epidermal growth factor receptor (EGFR), Akt family proteins and others [30]. Although further investigations are necessary to establish the precise intracellular mechanism of cisplatin on MAPK regulation in endothelial cells, our data demonstrated an enhancement of p38 MAPK and JNK phosphorylation in primary culture of HUVEC within 6 h after drug exposure. However, in agreement with previous observations [31, 32], we found that ERK phosphorylation remained unchanged in cisplatin-treated HUVEC, indicating a possible cell-type specific activation of MAPK cascades by cisplatin.

A number of studies showed that several signaling pathways controlling cell migration converge at MAPK cascades and many target molecules of MAPK signaling pathways are proteins involved in cell migration. MAPK family members regulate phosphorylation of myosin light-chain kinase, paxillin, calpain, microtubule associated protein and other molecules that are essential for cell migration [5]. In addition, MAPK activation has been reported to have variable effects on MMP2 and MMP9 regulation. MMP9 gene expression is regulated by ERK, p38 MAPK and JNK in human tracheal smooth muscle cells [33], while JNK regulates MMP2 expression in microvascular endothelial cells [34]. Although in the present study we showed that endothelial cell migration was partially abolished by p38 MAPK and JKN inhibitors, revealing that both MAPK family members are key mediators of endothelial cell migration, as previously described by other authors [4, 35], inhibition of p38 MAPK and JNK downstream cascades had no effect on MMP2 activity in HUVEC. In conclusion, our findings suggest that cisplatin effect on endothelial cell migration and MMP2 activity occurs through downstream mechanisms which are not related to MAPK family member activation.

Acknowledgements

This work was supported by Grant BFU2004-03268/BFI from the Ministerio de Educación y Ciencia/FEDER. We thank Ms. Irene González for the technical assistance for cell culture.

References

- 1 Bland ML, Desclozeaux M, Ingraham HA: Tissue growth and remodeling of the embryonic and adult adrenal gland. *Ann N Y Acad Sci* 2003;995:59-72.
- 2 Li S, Huang NF, Hsu S: Mechanotransduction in endothelial cell migration. *J Cell Biochem* 2005;96:1110-1126.
- 3 Yamaguchi H, Wyckoff J, Condeelis J: Cell migration in tumors. *Curr Opin Cell Biol* 2005;17:559-564.
- 4 Lamalice L, Le Boeuf F, Huot J: Endothelial cell migration during angiogenesis. *Circ Res* 2007;100:782-794.
- 5 Huang C, Jacobson K, Schaller MD: MAP kinases and cell migration. *J Cell Sci* 2004;117:4619-4628.
- 6 Xia Y, Karin M: The control of cell motility and epithelial morphogenesis by Jun kinases. *Trend Cell Biol* 2004;14:94-101.
- 7 Mehta PK, Griendling KK: Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol* 2007;292:C82-97.
- 8 Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T: Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem* 2003;253:269-85.
- 9 Yan C, Boyd DD: Regulation of matrix metalloproteinase gene expression. *J Cell Physiol* 2007;211:19-26.
- 10 Nagase H, Visse R, Murphy G: Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res* 2006;69:562-573.
- 11 Page-McCaw A, Ewald AJ, Werb Z: Matrix metalloproteinases and the regulation of tissue remodeling. *Nat Rev Mol Cell Biol* 2007;8:221-233.
- 12 Haas TA: Endothelial cell regulation of matrix metalloproteinases. *Can J Physiol Pharmacol* 2005;83:1-7.
- 13 Sluijter JP, de Kleijn DP, Pasterkamp G: Vascular remodeling and protease inhibition-bench to bedside. *Cardiovasc Res* 2006;69:595-603.
- 14 Deryugina EI, Quigley JP: Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Rev* 2006;25:9-34.
- 15 Fuertes MA, Castilla J, Alonso C, Pérez JM: Cisplatin biochemical mechanism of action: from cytotoxicity to induction of cell death through interconnections between apoptotic and necrotic pathways. *Curr Med Chem* 2003;10:257-266.
- 16 Wang G, Reed E, Li QQ: Molecular basis of cellular response to cisplatin chemotherapy in non-small cell lung cancer. *Oncol Rep* 2004;12:955-965.
- 17 Havelka AM, Berndtsson M, Olofsson MH, Shoshan MC, Linder S: Mechanisms of action of DNA-damaging anticancer drugs in treatment of carcinomas: is acute apoptosis an "off-target" effect? *Mini Rev Med Chem* 2007;7:1035-1039.
- 18 Jaffe EA, Nachman RL, Becker CG, Minick CR: Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J Clin Invest* 1973;52:2745-2756.
- 19 Montiel M, Pérez de la Blanca E, Jiménez E: Angiotensin II induces focal adhesion kinase/paxillin phosphorylation and cell migration in human umbilical vein endothelial cells. *Biochem Biophys Res Commun* 2005;327:971-978.
- 20 Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-254.
- 21 Cepeda V, Fuertes MA, Castilla J, Alonso C, Quevedo C, Pérez JM: Biochemical mechanisms of cisplatin cytotoxicity. *Anticancer Agents Med Chem* 2007;7:3-18.
- 22 Price PM, Yu F, Kaldis P, Aleem E, Nowak G, Safirstein RL, Megyesi J: Dependence of cisplatin-induced cell death in vitro and in vivo on cyclin-dependent kinase 2. *J Am Soc Nephrol* 2006;17:2434-2442.
- 23 Yu M, Han J, Cui P, Dai M, Li H, Zhang J, Xiu R: Cisplatin up-regulates ICAM-1 expression in endothelial cell via a NF- κ B dependent pathway. *Cancer Sci* 2008;99:391-397.
- 24 Folkman J: Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995;1:27-31.
- 25 Zhang H, Kim JK, Edwards CA, Xu Z, Taichman R, Wang CY: Clusterin inhibits apoptosis by interacting with activated Bax. *Nat Cell Biol* 2005;7:909-915.
- 26 Morikawa T, Shibuya M, Sakai S, Kudo S: Effect of anticancer drugs on invasive capacity of human small-cell lung cancer cells in vitro. *Nippon Ika Daigaku Zasshi* 1995;62:320-328.
- 27 Ramer R, Eichele K, Hinz B: Upregulation of tissue inhibitor of matrix metalloproteinases-1 confers the anti-invasive action of cisplatin on human cancer cells. *Oncogene* 2007;26:5822-5827.
- 28 Sasanelli R, Boccarelli A, Giordano D, Laforgia M, Arnesano F, Natile G, Cardellicchio C, Capozzi MA, Coluccia M: Platinum complexes can inhibit matrix metalloproteinase activity: platinum-diethyl[(methylsulfinyl)methyl]phosphonate complexes as inhibitors of matrix metalloproteinases 2, 3, 9, and 12. *J Med Chem* 2007;50:3434-3441.
- 29 Xue M, March L, Sambrook PN, Jackson CJ: Differential regulation of matrix metalloproteinase 2 and matrix metalloproteinase 9 by activated protein C: relevance to inflammation in rheumatoid arthritis. *Arthritis Rheum* 2007;56:2864-2874.
- 30 Brozovic A, Osmak M: Activation of mitogen-activated protein kinases by cisplatin and their role in cisplatin-resistance. *Cancer Lett* 2007;251:1-16.
- 31 Losa JH, Parada Cobo C, Viniegra JG, Sánchez-Arevalo Lobo VJ, Ramón y Cajal S, Sánchez-Prieto R: Role of the p38 MAPK pathway in cisplatin-based therapy. *Oncogene* 2003;22:3998-4006.
- 32 Brozovic A, Fritz G, Christmann M, Zisowsky J, Jaehde U, Osmak M, Kaina B: Long-term activation of SAPK/JNK, p38 kinase and fas-L expression by cisplatin is attenuated in human carcinoma cells that acquired drug resistance. *Int J Cancer* 2004;112:974-985.
- 33 Liang KC, Lee CW, Lin WN, Lin CC, Wu CB, Luo SF, Yang CM: Interleukin-1 β induces MMP-9 expression via p42/p44 MAPK, p38 MAPK, JNK, and nuclear factor- κ B signaling pathways in human tracheal smooth muscle cells. *J Cell Physiol* 2007;211:759-770.
- 34 Ispanovic E, Haas TL: JNK and PI3K differentially regulate MMP-2 and MT1-MMP mRNA and protein in response to actin cytoskeleton reorganization in endothelial cells. *Am J Physiol Cell Physiol* 2006;291:C579-588.
- 35 Yu J, Bian D, Mahanivong C, Cheng RK, Zhou W, Huang S: p38 Mitogen-activated protein kinase regulation of endothelial cell migration depends on urokinase plasminogen activator expression. *J Biol Chem* 2004;279:50446-50454.