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Mechanism of vascular endothelial growth factor expression mediated by cisplatin in human ovarian cancer cells

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Abstract

Cisplatin (CDDP) and its analogues are widely used for the treatment of a variety of human solid tumors. However, the molecular mechanism of its action remains to be understood. Vascular endothelial growth factor (VEGF) is a potent inducer of angiogenesis and is upregulated in many human cancers. In this study we demonstrated that CDDP-inhibited VEGF expression in human ovarian cancer cells. We found that CDDP inhibited the VEGF reporter activity in a dose-dependent manner, indicating that CDDP-inhibited transcriptional activation of VEGF. We also found that: (1) luciferase activity mediated by the VEGF reporter containing a mutation of the HIF-1 binding site was much lower than that of the reporter containing a wild-type HIF-1 binding site in ovarian cancer cells, thus confirming that HIF-1 is a major transcriptional regulator of VEGF expression; and that (2) CDDP greatly inhibited VEGF reporter activity containing the wild-type but not the mutant HIF-1 binding site. This result indicates that CDDP-inhibited VEGF transcriptional activation specifically by decreasing HIF-1 activity. Co-transfection of a dominant negative construct of HIF-1 inhibited VEGF reporter activity in ovarian cancer cells. CDDP-inhibited VEGF transcriptional activation specifically through the expression of HIF-1α, but not HIF-1β. We demonstrated that VEGF receptor KDR was expressed in ovarian cancer cells, and that CDDP-inhibited VEGF expression was linked with cellular apoptosis, which was rescued by VEGF treatment. These results suggest a novel mechanism of CDDP's anti-tumor activity in ovarian cancer cells via HIF-1 expression and VEGF transcriptional activation.

Keywords: Cisplatin; Ovarian cancer; HIF-1; VEGF; Angiogenesis

CDDP and its analogues are useful particularly in the treatment of late stage ovarian cancer and are part of the standard chemotherapy treatment for this disease [1]. The primary mechanism responsible for the anti-tumor activity of CDDP is thought to be the cross-linking of CDDP with genomic DNA and the induction of apoptosis [2–4]. However, the cellular response to DNA damage is complex and the specific mechanism that causes cell death remains to be elucidated [5].

Vascular endothelial growth factor (VEGF) is a potent inducer of angiogenesis both *in vivo* and *in vitro* [6–8]. Mutations of one or both alleles of the VEGF gene result in an embryonic lethal phenotype in mice due to inadequate vascularization, suggesting that VEGF is required for the early stages of vascular development [9,10]. VEGF expression is upregulated in a variety of human tumors, including ovarian tumors [11], and VEGF-stimulated angiogenesis seems to be required for this type of tumor [12,13]. The inhibition of VEGF expression in turn inhibited tumor angiogenesis *in vivo* and decreased tumor size in nude mice

Abbreviations: CDDP, cisplatin; VEGF, vascular endothelial growth factor; HIF-1, hypoxia-inducible factor 1; KDR, VEGF receptor 2.

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[14–16]. VEGF expression is also upregulated by the hypoxic conditions in tumor cells and the activation of oncogenes such as v-Src and Ras [17–19].

Hypoxia inducible factor 1 (HIF-1) is a heterodimeric helix–loop–helix transcription factor that consists of two subunits: HIF-1α and HIF-1β [20,21]. HIF-1β is constitutively expressed in cells, while HIF-1α stability is stimulated by hypoxia, growth factors, and several oncogenes [22]. HIF-1 has a number of transcriptional targets including VEGF [23–26].

Materials and methods

Reagents and cell culture. Cisplatin (CDDP) was obtained from Sigma (St. Louis, MO). The human ovarian cancer cell lines OVCAR-3 and A2780/CP70 were maintained in RPMI 1640 media supplemented with 10% fetal calf serum. All cells were cultured at 37 °C in a 5% CO_2 incubator.

DNA constructs. The dominant negative form of HIF-1, HIF-1α DN, was subcloned into pCEP4 vector (Invitrogen). The human VEGF reporter construct was constructed by inserting a 1.9 kb of the human VEGF gene promoter into the pGL2-basic vector. The pMAP11 WT VEGF reporter was constructed by PCR amplification of a fragment of the VEGF promoter from –985 to –939; the pMAP11 mutant was constructed by a three base pair substitution in the HIF-1 binding sequence in the pMAP11 WT VEGF reporter.

Immunoblot analysis. Aliquots of protein extracts were resolved in SDS/polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and analyzed using antibodies against HIF-1α and HIF-1β (Transduction Laboratories, Lexington, KY).

Preparation of RNA and Northern blots. Total cellular RNAs were isolated using RNA STAT-60 (Tel-Test Inc., Friendswood, TX), aliquots of 10 μg total RNAs were separated by electrophoresis in 2.2 M formaldehyde/0.9% agarose gel, transferred to a nylon membrane, and analyzed by Northern blots with [^{32}P]dATP-labeled human VEGF cDNA and actin cDNA.

Transient transfection and luciferase assays. The cells were transfected with the plasmid DNAs using LipofectAMINE (Invitrogen, Carlsbad, CA). After the transfection, the cells were cultured. The cells were lysed,

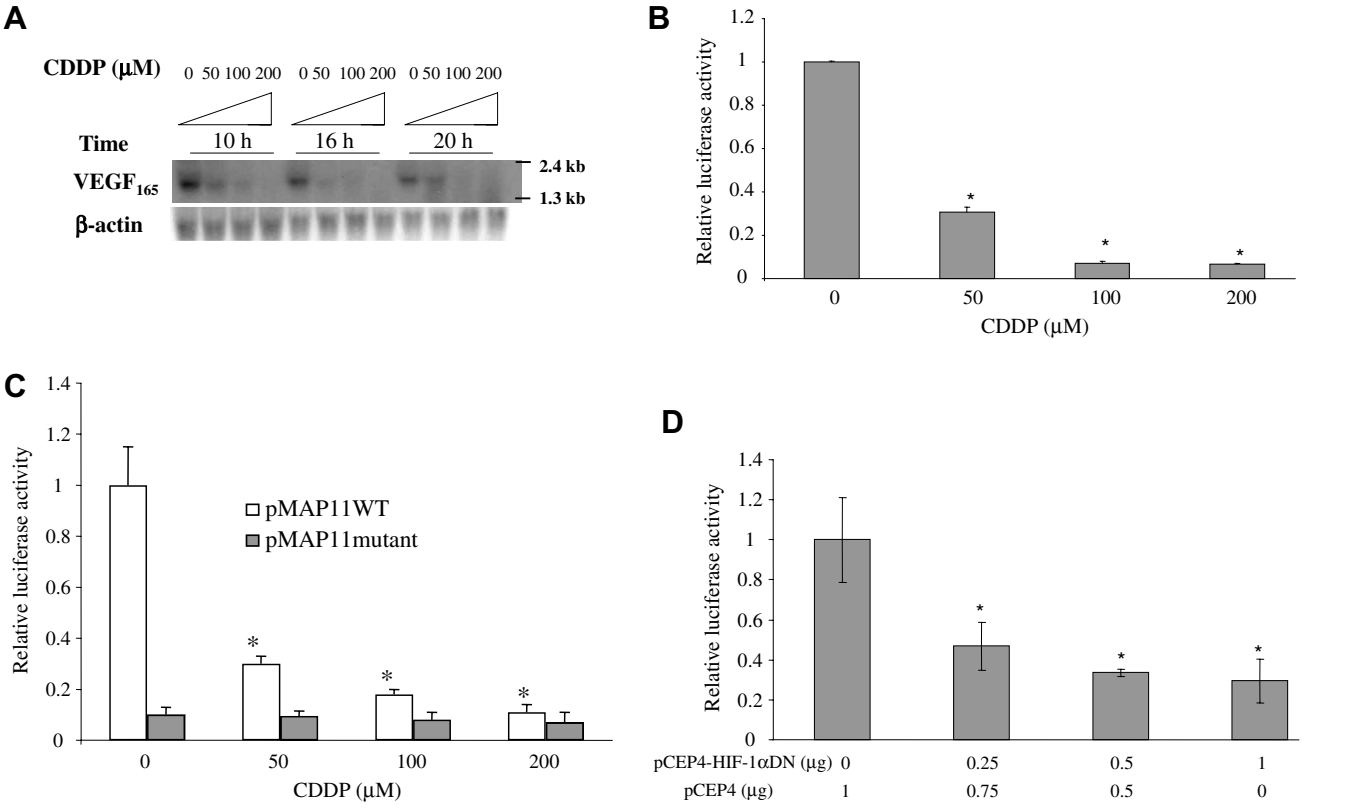

Fig. 1. Effects of CDDP on VEGF mRNA expression and transcriptional activation through the HIF-1 DNA binding site in the promoter. (A) A2780/CP70 cells were treated in the absence or presence of CDDP (50, 100 or 200 μM) for 10, 16, or 20 h as indicated. Total cellular RNAs were prepared, and used for Northern blot analysis. (B) A2780/CP70 cells were co-transfected with pCMV-β-gal plasmid and a human VEGF reporter, pVEGF-Luc, containing a 1.9 kb human VEGF promoter fragment inserted upstream of firefly luciferase. After the transfection, the cells were cultured for 12 h, followed by the treatment of CDDP at the concentrations indicated for 24 h. Relative luciferase activity was determined by the ratio of luciferase to β-gal activity, and normalized to the value obtained from A2780/CP70 cells in the absence of CDDP. The figure shows mean ± SD of relative luciferase activities for each group. *indicates that the relative luciferase activity was significantly different when compared to that of the control ($p < 0.01$). (C) A2780/CP70 cells were co-transfected with pCMV-β-gal and a VEGF promoter containing a 46 bp functional human VEGF promoter with the HIF-1 binding site, pMAP11WT reporter, or the pMAP11mutant which contained 3-bp mutation of the HIF-1 binding site. Relative luciferase activity was obtained and normalized to that from the cells transfected with the pMAP11WT and treated with vehicle only. *indicates that the relative luciferase activity was significantly different when compared to that of the pMAP11WT control in the absence of CDDP ($p < 0.01$). (D) Effect of HIF-1α on the VEGF reporter activity. A2780/CP70 cells were co-transfected with 0.5 μg pMAP11WT reporter and a HIF-1α dominant negative construct in pCEP4 vector at 0, 0.25, 0.5, and 1 μg. Relative luciferase activity was normalized to that from the parental expression vector pCEP4 only. The figure shows mean ± SD of relative luciferase activities for each group. *indicates that the relative luciferase activity was significantly different when compared to that of the control ($p < 0.01$).