Bcl-2 Promotes Invasion and Lung Metastasis by Inducing Matrix Metalloproteinase-2

Jihyung Choi,1 Kyusam Choi,2 Etty N. Benveniste,4 Young-Sook Hong,1 Je-Ho Lee,2 Jhingook Kim,3 and Kyoungsook Park

1Department of Biochemistry, College of Medicine, Ewha Womans University; 2Molecular Therapy Research Center and Department of Thoracic Surgery, Sungkyunkwan University School of Medicine, Samsung Medical Center, Ilwon-dong, Kangnam-ku, Seoul, Korea; and 3Department of Cell Biology, The University of Alabama at Birmingham, Birmingham, Alabama

Abstract

Bcl-2 is involved in the progression of human malignancies, but the precise role and mechanism of Bcl-2 for tumor invasion and metastasis remains unclear. In this study, we have investigated the role and mechanism of Bcl-2 on tumor cell invasion and metastasis by using Bcl-2 overexpressing non–small cell lung cancer cells. Matrix metalloproteinases (MMPs) are important proteins involved in the processes of tumor invasion and metastasis. In vitro Matrigel invasion assays showed that Bcl-2 overexpression increased tumor cell invasion by 15-fold. Moreover, Bcl-2 overexpression enhanced in vivo lung metastasis by 4-fold. Consistent with its effect on invasion and metastasis, Bcl-2 overexpression induced not only MMP-2 mRNA and its protein expression, but this also activated the pro-MMP-2 protein to its active form. To explore the induction mechanism of MMP-2 by Bcl-2, we investigated the effects of Bcl-2 overexpression on MMP-2 transcriptional regulation. Nuclear run-on assays showed a 6-fold increase in the transcription rate of MMP-2 mRNA in the Bcl-2 transfectants (H157/Bcl-2) compared with that of the H157/vector control cells (H157/C). Overexpression of Bcl-2 induced the nuclear transcription factor activator protein 1 family, including the c-Jun, JunD, c-Fos, FosB, and Fra-1 proteins. Reporter assays combined with deletion mutagenesis analysis and gel shift assays showed the involvement of activator protein 1 in the activation of MMP-2 promoter activity by Bcl-2. Taken together, we have shown that Bcl-2 promotes tumor invasion and lung metastasis by inducing MMP-2 gene expression through the combined action of transcriptional and posttranslational mechanisms. (Cancer Res 2005; 65(13): 5554-60)

Introduction

Bcl-2 protects cells from apoptosis induced by a variety of apoptotic stimuli (1–4). Overexpression of Bcl-2 was reported to be correlated with the progression of various human malignancies (5–7). In addition to its well-established role in promoting cell survival, recent studies have revealed that Bcl-2 also has other important functions including regulation of the cell cycle, modulation of cell differentiation, and the regulation of gene expression (4). Moreover, Bcl-2 has been found to be involved in tumor metastasis and angiogenesis (8–10).

Tumor metastasis occurs by a series of steps including vessel formation, cell attachment, invasion, and cell proliferation, and the regulation of metastasis is extremely complicated (11, 12). Degradation of basement membranes and the stromal extracellular matrix are crucial steps for tumor invasion and metastasis. The matrix metalloproteinases (MMPs) are a family of human zinc– dependent endopeptidases responsible for degradation of the extracellular matrix (13, 14). Among them, the gelatinases (MMP-2 and MMP-9) efficiently degrade native collagen types IV and V, fibronectin, entactin, and elastin. Therefore, these proteases are believed to be of crucial importance in the processes that require disrupting the basement membrane such as tumor invasion and metastasis (15).

In this study, we investigated the role of Bcl-2 for the invasion and metastatic potential of human non–small cell lung cancer (NSCLC) cells that overexpress Bcl-2. We generated stable Bcl-2 overexpressing human NSCLC cells (H157/Bcl-2 cells) that expressed different levels of Bcl-2 protein and we addressed the question as to whether overexpression of Bcl-2 was capable of increasing the in vitro invasion capability and the in vivo lung metastatic potential of NSCLC cells. The promotion of the invasiveness and metastatic potential of NSCLC cells by Bcl-2 showed a strong correlation with the induction and activation of MMP-2. Our findings showed the induction of transcription factor activator protein 1 (AP-1) and importance of its binding site for the activation of the MMP-2 promoter by Bcl-2. These results provide a novel mechanism to explain how Bcl-2 may promote tumor invasion and metastasis.

Materials and Methods

Cell culture and transfection. The human NSCLC cell line, NCI-H157, was obtained from the American Type Culture Collection (Rockville, MD), and the cells were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum. The Bcl-2 expression vector (pCI-neo/Bcl-2) was prepared by inserting Bcl-2 cDNA into the EcoRI cloning site of the pCI-neo vector (Promega, Madison, WI). The pCI-neo vector–transfected H157 cells (H157/Bcl-2 cells) and the pCI-neo vector–transfected cells (H157/C) were selected by growth in the presence of G418 and then analysis for expression of Bcl-2 by Northern blotting, immunoblotting, and immunocytochemistry.

Northern blotting. Total RNA was isolated using TRIzol (Life Technologies, Grand Island, NY) according to the manufacturer’s instructions. Ten micrograms of total RNA were separated on a 1.2% agarose gel, transferred onto a Hybond-N membrane (Amersham, Buckinghamshire, United Kingdom). The blot was hybridized with [α-32P]dCTP-labeled cDNA probe as previously described (16).

Immunoblotting. Subconfluent cells (80% confluent) were washed with PBS, and then incubated in serum-free RPMI 1640 for 12 hours. The serum-free medium was collected and then concentrated 50-fold by using a Centricon-30 device (Amicon, Beverly, Mass) and then analyzed by immunoblotting

Note: J. Choi and K. Choi contributed equally to this work. Requests for reprints: Kyungsook Park, Molecular Therapy Research Center, Sungkyunkwan University, Samsung Medical Center, Annex 8F, 50 Ilwon-dong, Kangnam-ku, Seoul, Korea. Phone: 82-2-3410-3638; Fax: 82-2-3410-3649; E-mail: hannah05@dreamwiz.com.

©2005 American Association for Cancer Research.
Promotion of Invasion and Metastatic Potential by Bcl-2

37°C, the cells attached to the lower chamber were fixed with methanol, stained with 0.1% crystal violet, and then counted under a light microscope.

Assay of spontaneous metastasis. Female BALB/c nude (nu/nu) mice (5 weeks old) were purchased from Charles River Laboratory (Japan), and housed under pathogen-free conditions. The Animal Care and Use Committee of Samsung Biomedical Research Institute approved the animal experiments described herein. Each experiment group included 10 animals. For the experimental metastatic assays, parental and transfectant cells (1 × 10⁶ cells resuspended in 100 μL of PBS) were injected into the footpad of each nude mouse. Five weeks after the injection, the legs of mice with tumors were amputated. The mice were sacrificed 3 months after the amputation and their lungs were removed and prepared for immunohistochemistry. The metastatic nodules in the lungs were counted. The results were analyzed by the Mann-Whitney U test for statistical significances. Differences were considered significant at P values < 0.05 (two-tailed).

RT-PCR. Total RNA was used to prepare cDNA by using SuperScript II reverse transcriptase (Life Technologies), and then subsequent PCR was done using Taq polymerase (Roche, Indianapolis, IN). The PCR conditions were an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute, and a final extension step was done for 10 minutes at 72°C. The MMP-2 sequence was amplified with the forward primer (5'-ggacacactaaggaagatca-3') and the reverse primer (5'-gcgcatctcagcagcc-3'). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control to normalize for the cDNA abundance. The RT-PCR assays were all done in triplicate.

Nuclear run-on transcription analysis. The nuclear run-on assay was done as reported previously (16). After pelleting the nuclei, 200 μL of 2× reaction buffer [10 mmol/L Tris-HCl (pH 8.0), 5 mmol/L MgCl₂, 0.3 mol/L KCl, 1 mmol/L ATP, CTP, and GTP, 5 mmol/L DTT, and 100 μCi (α-32P)ATP] was added to the nuclei sample and then allowed to incubate for 30 minutes at 30°C. Afterwards, the nuclei were treated with DNase I (24 units) in 0.5 mmol/L NaCl, 50 mmol/L MgCl₂, 2 mmol/L CaCl₂, and 10 mmol/L Tris-HCl (pH 7.4) for 15 minutes at 37°C. The radioactively labeled RNAs were extracted with phenol-chloroform and purified on a QiA shredder spin column (Qiagen, Hilden, Germany); then hybridized to human MMP-2 or GAPDH cDNA that was immobilized on a nylon membrane (Hybond-XL, Amersham). The blots were washed, treated with RNase to digest the nonhybridized RNA, and autoradiographed. Specific hybridization to MMP-2 was quantified using a PhosphorImager and then normalized to that of GAPDH.

Transient transfection and luciferase assays. The human MMP-2 promoter luciferase reporter constructs (designated as −1,629 MMP2 LUC, −1,050 MMP2 LUC, −562 MMP2 LUC, and −546 MMP2 LUC) were described previously (18). AP-1 internal deletion mutant of pGL2-MMP-2-Luc was prepared by using a site-directed mutagenesis method according to the manufacturer's protocol (Stratagene, La Jolla, CA) using the oligonucleotides (5'-gctcttcaggtctcagctcttcttccaggaagccttc-3' and 5'-gaaggtgtctgc- gaagagctgagctgacgagg-3') to introduce the AP-1 site deletion into the MMP-2 promoter. Stable H157/Bcl-2 transfected cells at 70% confluency were transiently transfected with the indicated reporter construct. As an internal control to correct for variations in transfection efficiency, 20 ng of pRL-TK (Promega), was cotransfected. The transfections were done using Effectene transfection reagent (Qiagen) according to the manufacturer's instructions. Luciferase activity was measured using a dual luciferase reporter assay system (Promega) according to the manufacturer's instructions, and this was normalized for the Renilla luciferase activity to correct for variations in the transfection efficiency.

Electrophoretic mobility shift assay. Electrophoretic mobility shift assays were done as previously reported (16). In brief, each reaction contained 4 μg of poly (dI-dC), 10 mmol/L Tris-HCl (pH 7.5), 50 mmol/L NaCl, 1 mmol/L DTT, 1 mmol/L EDTA, 50% glycerol, 5,000 to 6,000 cpm 32P-labeled AP-1 probe, and μg of nuclear extract. After incubation for 30 minutes at room temperature, the reactions were electrophoresed on 5% native polyacrylamide gels using 0.25× Tris-borate EDTA buffer using Centriplus (Amicon, Bedford, MA). Ten micrograms of each concentrated conditioned medium was subjected to 10% SDS-PAGE followed by incubation with the indicated antibodies.

Gelatin zymography. Activity of the MMP-2 secreted by the NSCLC cell lines was analyzed by gelatin zymography. The conditioned medium was concentrated as described in immunoblotting and then 2 μg of each concentrated medium was subjected to 10% SDS-PAGE containing 0.1% gelatin (wt/vol) as previously described by Wick et al. (17).

Matrigel invasion assay. Tumor cell invasion was measured with a Biocoat Matrigel invasion chamber (Becton Dickinson Labware, Bedford, MA) according to the manufacturer's instructions. Cells (5 × 10⁴) in 0.5 mL of serum-free medium were seeded onto the upper chamber, and 0.75 mL of complete growth medium containing 10% fetal bovine serum was added to each well in the lower chamber. After incubation for 72 hours at

![Figure 1. Establishment of Bcl-2 overexpressing human H157 NSCLC cells. A, overexpression of Bcl-2 protein in H157/Bcl-2 stable transfectants. Whole-cell lysates from the H157/Bcl-2 transfectants and the H157/C cell transfectants with control vector were subjected to immunoblot analysis with anti-Bcl-2 antibody as described in Materials and Methods. B, Northern blot analysis of the representative Bcl-2 overexpressing transfectant. Ten micrograms of total RNA were subjected to Northern blot analysis with 32P-labeled Bcl-2. β-Actin probe was used as a control for RNA loading. H157, parental cell line; H157/C, pCl-neo vector–transfected cells; H157/Bcl-2, pCl-neo-Bcl-2–transfected cells (clone 9). The expression of Bcl-2 protein was determined by immunoblot analysis with anti-Bcl-2 antibody as described in Materials and Methods. C, immunocytochemistry for the expression of Bcl-2 in the H157/Bcl-2 stable transfectant (clone 9). The cells were plated onto four-chamber slides and then processed for immunocytochemistry.](image-url)
(22.5 mmol/L Tris borate and 0.5 mmol/L EDTA). AP-1 wild-type oligonucleotide probe was prepared by end-labeling of the annealed double-stranded oligonucleotides with T4 polynucleotide kinase and [$\gamma$-$^{32}$P]ATP. For the binding competition experiments, double-stranded wild-type (5'-cggcttgatgactcagccggaa-3') or mutant (5'-cggcttgatgacttggccggaa-3') AP-1 oligonucleotides were added at a 100-fold molar excess as a cold competitor 15 minutes before the addition of [$^{32}$P]-labeled probe. Antibody interference assays were carried out by incubation with anti-c-Jun antibody for 20 minutes before the addition of the $^{32}$P-labeled probe.

Results

Generation of Bcl-2 overexpressing human NSCLC H157 clones. To examine the role of Bcl-2 on the invasiveness and metastatic potential of human lung cancer cells, the H157 cells that showed a low level of Bcl-2 protein were transfected with either the pCl-neo/Bcl-2 expression vector or pCl-neo vector. We were able to generate stable H157/Bcl-2 clones that expressed different levels of Bcl-2 protein (Fig. 1A). The representative lysates of the H157/Bcl-2 transfectants (clones 3, 4, 5, 6, 7, 8, 9, and 11) that expressed high levels of Bcl-2 protein revealed dramatic induction of Bcl-2 mRNA and protein compared with their H157 parental line or the vector control transfectant (H157/C), as is shown in Fig. 1B. Immunocytochemistry with anti-Bcl-2 antibody further confirmed the expression of Bcl-2 in representative cells of H157/Bcl-2 transfectants (Fig. 1C).

Bcl-2 overexpression increases the in vivo and in vitro invasive potential of Bcl-2 overexpressing H157 cells. In order to evaluate whether the Bcl-2 contributes to invasive behavior of the H157 NSCLC cells, we examined the invasive potential of a Bcl-2 transfectant (clone 9) by using an in vitro Matrigel chamber assay. In contrast to the H157 and H157/C cells, which showed low penetration of the Matrigel-coated membrane, H157/Bcl-2 cells showed a high level of penetration (Fig. 2A, top). The number of H157/Bcl-2 cells that passed through the Matrigel-coated membrane was increased 15-fold compared with that of H157 or H157/C cells (Fig. 2A, bottom). This finding indicates that overexpression of Bcl-2 can enhance in vitro tumor invasion for NSCLC cells. To further confirm the dramatic increase in the invasive potential of the Bcl-2 transfectant cells in vivo, nude mice were injected with H157/C cells or H157/Bcl-2 cells, and then mice were examined for the formation of pulmonary metastatic nodules. Although lung metastatic nodules were observed in 8 out of 10 mice that had been injected with H157/Bcl-2, only 2 of 10 mice injected with vector control transfectant (H157/C) showed lung metastasis (Table 1). Furthermore, the H157/Bcl-2 cells formed more pulmonary metastatic nodules than did the H157/C cells (Fig. 2B). There was no significant difference in the size of the primary tumors formed in the legs of the mice (Table 1). The expression of Bcl-2 was then confirmed by immunohistochemistry. Strong staining of Bcl-2 protein was observed in the...
lung tumor tissues derived from the metastatic tumors from animals injected with the Bcl-2 transfectant cells (Fig. 2C). Taken together, the Bcl-2 overexpressing H157/Bcl-2 transfectant cells shows a much higher metastatic potential than did the H157/C cells in vitro and in vivo.

**Bcl-2 increases MMP-2 gene transcription and its activity in the H157/Bcl-2 cells.** To explore whether the increased invasiveness and metastatic potential of the H157/Bcl-2 transfectant cells was associated with MMP induction, RT-PCR was done with gene-specific MMP primer sets to screen for a potential candidate (data not shown). Among the MMPs that were examined, MMP-2 expression was dramatically induced in the H157/Bcl-2 transfectant (Fig. 3A). To further confirm the induction of MMP-2 mRNA by Bcl-2, Northern blot analysis was done. Expression of the 3.1 kb MMP-2 transcript was induced in the H157/Bcl-2 transfectant cells compared with the H157 or H157/C cells (Fig. 3B, top). Consistent with this result, we detected the induction of MMP-2 protein in the H157/Bcl-2 transfectant cells (Fig. 3B, bottom). To investigate whether the induction of MMP-2 via Bcl-2 overexpression can occur at the transcriptional level by increasing the transcriptional rate, nuclear run-on assays were done with nuclei that were prepared from both H157/C and H157/Bcl-2 cells. The transcription rate of the MMP-2 gene was 6-fold greater in the H157/Bcl-2 cells than the transcription rate of the MMP-2 gene in the H157/C cells (Fig. 3C). Next, we examined whether the overexpression of Bcl-2 could lead to an increase in MMP-2 activity by using gelatin zymography on the concentrated serum-free conditioned medium. There was a 5-fold increase in the level of secreted 72 kDa gelatinase A (latent MMP-2) in the H157/Bcl-2 cells compared with that in the H157 or H157/C cells (Fig. 3D). Most importantly, the 66 kDa active form of MMP-2 that was derived from the proteolytic removal of the latent MMP-2 was detected only in H157/Bcl-2 cells. These results show that Bcl-2 induces steady-state mRNA levels of MMP-2 through the activation of the transcription rate, and it can also activate its latent form.

**Bcl-2 induces the activation of the human MMP-2 promoter and the AP-1 binding site is critical for the Bcl-2-mediated MMP-2 activation.** To investigate whether Bcl-2 overexpression has a direct effect on human MMP-2 promoter activity, H157/Bcl-2 transfectants showing different levels of Bcl-2 expression were transfected with a human MMP-2 luciferase reporter construct (−1,629 MMP2 LUC; Fig. 4A). Overexpression of Bcl-2 resulted in an increase in the MMP-2 promoter activity in a dose-dependent manner (Fig. 4B). Statistical analysis revealed a strong correlation between the level of Bcl-2 expression and the human MMP-2 promoter activity (Pearson's coefficient = 0.993; P < 0.01). To further explore the transcriptional regulatory elements and the transcription factors involved in human MMP-2 promoter activation by Bcl-2, the H157/Bcl-2 cells were transiently

---

**Table 1. Effect of Bcl-2 expression on the metastatic potential of NSCLC cells**

<table>
<thead>
<tr>
<th>Cell line*</th>
<th>Weight of the primary tumor (g)</th>
<th>Incidence of lung metastasis (%)</th>
<th>Average no. of tumor nodules in the lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>H157/C</td>
<td>1.42 ± 1.32†</td>
<td>20</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>H157/Bcl-2</td>
<td>1.38 ± 0.60‡</td>
<td>80*</td>
<td>3.2 ± 0.3**</td>
</tr>
</tbody>
</table>

*Cells (1×10⁶/mouse) were injected into the footpad of nude mice.
†The weight of the leg with the tumor was determined 5 weeks after injection.
‡The mice were killed 3 months after the amputation of their legs with tumor.
Mean ± SD.
*P > 0.5.
**P = 0.023 (Fisher's exact test).
**P < 0.05 (two-tailed) in comparison with H157/C cells.*

---

**Figure 3.** Induction and activation of MMP-2 by Bcl-2. A, RT-PCR analysis of MMP-2 expression in H157/Bcl-2 cells (clone 9). β-Actin was used as a control for loading. B, induction of MMP-2 mRNA and protein in H157/Bcl-2 cells (clone 9). Total RNA was isolated and subjected to Northern blot analysis with an MMP-2 cDNA probe. C, Bcl-2 increases the transcriptional rate of MMP-2. Nuclear run-on assays of MMP-2 transcription were done using nuclei from the H157 and H157/Bcl-2 cells (clone 9) as described in Materials and Methods. After in vitro transcription in the presence of [γ-32P]UTP, equal amounts of the labeled transcripts were hybridized to MMP-2 or GAPDH cDNA that was immobilized on a nylon membrane. Specific hybridization to MMP-2 was quantified using a PhosphorImager and was normalized to that of GAPDH. D, activation of the MMP-2 protein by Bcl-2. Gelatin zymography of the MMP-2 protein in serum-free conditioned medium showed activation of MMP-2 protein in the Bcl-2 transfectants (clone 9).
Discussion

Bcl-2 can contribute to neoplastic cell growth by enhancing the survival of tumor cells through the inhibition of apoptosis (3). In addition to its antiapoptotic property, Bcl-2 was also shown to enhance the invasive properties and migration of several cancer cells by altering the expression level of a set of MMPs and their inhibitors (9, 17). In this study, we have shown the following: (a) the overexpression of Bcl-2 in H157 cells, which have a low spontaneous metastasis rate, can promote in vitro tumor cell invasion as well as significantly enhancing the in vivo spontaneous metastatic potential; (b) overexpression of Bcl-2 results in a significant increase in MMP-2 expression and secretion, as well as increasing its activity; (c) a significant correlation between the level of Bcl-2 and the increased MMP-2 promoter activity was found; (d) induction of MMP-2 by Bcl-2 involves transcriptional activation of MMP-2 through the AP-1 binding site with the induction of the AP-1 proteins. When taken together, these results further support that the increased expression and activation of MMP-2 by Bcl-2 would influence tumor invasion and metastasis in NSCLC cells.

Conversion of the latent MMPs to their active form involves the proteolytic removal of an amino terminal domain of the latent MMPs, and the latent and activated forms can be resolved by their size (14). Although the “latent” MMP species also display gelatinolytic activity because of the artificial conditions of the zymographic process, this 72 kDa MMP-2 latent species are known to be inactive under physiologic conditions (19, 20). Thus, we examined whether the induced MMP-2 is functionally active for promoting tumor invasion and metastasis. Our gelatin zymography analysis showed not only a 5-fold induction of the 72 kDa latent gelatinase A (the latent MMP-2) in the H157/Bcl-2 cells, but it also showed the induction of an active 66 kDa MMP-2 in only the H157/Bcl-2 cells (Fig. 3D). Taken together, the induced MMP-2 activity can contribute to the enhanced invasiveness and metastatic potential of H157/Bcl-2 NSCLCs in vitro and in vivo and thus, lead to the malignant phenotype of Bcl-2 overexpressing NSCLC cells.

Investigations by other laboratories showed that MMP-2 was induced by transforming growth factor-1 through both transcription and an increased mRNA stability in human gingival fibroblasts (21), and MMP-2 was also induced by transforming growth factor-1 through changes in both the mRNA levels and extracellular processing in human fibrosarcoma cells (22). The significance of p53, Sp1, Sp3, and AP-2 for the regulation of MMP-2 gene expression has been previously shown (18, 23). In addition, lipopolysaccharide can also directly enhance angiogenesis by inducing MMP-2 activation that is mediated through an nuclear factor κB pathway in endothelial cells (24). Our results in this study suggest that the transcriptional activity of the MMP-2 promoter is dependent upon the level of Bcl-2 expression (Fig. 4B). Several members of the MMP protein family are known to be inducible and there is a high degree of cotransfected with a pRL-TK renilla construct (internal control) and with the wild-type (−1,629 MMP2 LUC) or they were transiently cotransfected with three deletion mutant constructs (−1,050 MMP2 LUC, −562 MMP2 LUC, and −546 MMP2 LUC), respectively (Fig. 5A). These deletion mutants lack the AP-1 binding site located at −1,265 in the human MMP-2 promoter. The MMP-2 promoter activity was reduced 2-fold with the deletion of the AP-1 binding site, and this suggests that the AP-1 binding site was necessary for the transcriptional regulation of the MMP-2 by Bcl-2 (Fig. 5A). To verify that the transcription factor AP-1 proteins can indeed be induced by Bcl-2 overexpression, immunoblot analysis was done using nuclear extracts prepared from H157/C and H157/Bcl-2 cells. Twenty micrograms of the nuclear extracts prepared from the H157/C and H157/Bcl-2 cells were immunoblotted with antibodies against the different members of the AP-1 family as indicated (Fig. 5B). Dramatic increases in the induction of AP-1 family proteins, especially JunD, FosB, c-Jun, and c-Fos, were observed in the nuclear extract from the H157/Bcl-2 transfectant. The importance of the AP-1 binding site was further confirmed by deleting the AP-1 site in the context of the natural MMP-2 promoter, pGL2MMP-2. Deletion of AP-1 binding site, pGL2MMP-2 (ΔAP-1), abrogated activation of MMP-2 promoter activity by the Bcl-2 protein (Fig 5C). This dramatic effect was comparable to that obtained with the 5′ AP-1 deletion mutants, as shown in Fig. 5A, and this suggests the importance of the AP-1 binding site for the activation of the MMP-1 promoter by Bcl-2 protein in human NSCLC cells. Next, to examine whether the AP-1 binding site can be bound by AP-1, electrophoretic mobility shift assays were done with an AP-1 oligonucleotide probe (Fig. 5D). We observed the dramatic induction of the DNA-protein complex in the nuclear extract prepared from the H157/Bcl-2 cells (lane 2) compared with that prepared from the H157/C cells (lane 6). Although the wild-type AP-1 oligonucleotides (lane 4) were capable of outcompeting the DNA-protein complex formation, the mutant AP-1 oligonucleotides were not (lane 3). In addition, the antibody interference assay revealed the presence of c-Jun protein in this DNA-protein complex (lane 5).

![](https://cancerres.aacrjournals.org/-/media/images/8904.jpg)
conservation in the regulatory elements within the promoter regions of these genes (25). Sequence analysis of the human MMP-2 promoter has revealed a number of potential *cis*-acting regulatory elements including p53, AP-1, Ets-1, the polyoma enhancer activator, and c-myc (23). The cell-specific and stimulus-specific expression and regulation of the MMP-2 gene have previously been reported (18, 23, 26, 27).

The two most important binding sites found within the promoter regions of the MMP-2 gene are the AP-1 and the polyoma enhancer activator sites (Fig. 5A).5 When the AP-1, Ets-1, and c-myc binding sites were deleted, the activity of the MMP-2 promoter was decreased by 2.5-fold compared with the wild-type MMP-2 promoter (Fig. 5A). Consistent with this luciferase activity, we have observed a significant induction of AP-1 family proteins in the extract of the H157/Bcl-2 cells (Fig. 5B). These findings suggest the significance of the AP-1 family in the positive regulation of MMP-2 promoter activity. These results are consistent with other reports that have shown that AP-1 acts as a positive regulator for the promoter activities of the other MMPs (28–30). In contrast, Koo et al. reported that Bcl-2 expression suppressed the activation of c-Jun NH2-terminal kinase in Jurkat T-cells treated with cisplatin (31). In addition, the stable expression of Bcl-2 in fibroblasts inhibited activation of c-Jun NH2-terminal kinase by interleukin-1β (32). Our results with the AP-1 binding site deletion in the context of the full-length MMP-2 promoter shows the critical role

---

5 J. Choi and K. Park, unpublished data.
of the AP-1 site in the MMP-2 promoter. Our preliminary data also suggests the involvement of polyoma enhancer activator in the transcriptional regulation of MMP-2 by Bcl-2 (data not shown). Crawford et al. showed that the expression of polyoma enhancer activator, in conjunction with the accumulation of β-catenin in gastrointestinal tumors, leads to the up-regulation of MMP-2 gene transcription (33). Further detailed analysis of the MMP-2 promoter would provide information on the positive and negative modulation of the activity of several transcription factors in the context of H157 cells.

Recent findings by Feng et al. (34) have shown how Bcl-2 might regulate gene expression. They showed that the overexpression of Bcl-2 up-regulated the expression of c-jun and c-fos, as well as enhanced the DNA binding activity and transcriptional activity of AP-1. The Bcl-2-mediated increase in AP-1 activity further affected the downstream genes containing AP-1 binding sites. These results provide a mechanism to explain how Bcl-2 may regulate the expression of other genes. Consistent with these results, our findings suggest that Bcl-2 can directly up-regulate MMP-2 gene expression and activity for promoting the invasion and tumor metastatic potential of human NSCLC cells by directly inducing AP-1 DNA-binding activity and its transcriptional activity.

In conclusion, our data show that Bcl-2 promotes both the invasion and metastatic potential of human NSCLC cells in vitro and in vivo through the regulation of MMP-2 gene expression and activity. A dramatic increase in the expression and activity of MMP-2 promotes invasion and metastasis in NSCLC cells. The remarkable induction of MMP-2 by Bcl-2 involves the transcriptional activation of the MMP-2 promoter, and this is mediated through the AP-1 binding site and the induction of AP-1 factors. Our findings should provide a clue for the development of novel therapeutic strategies and for the improved survival of patients with advanced lung cancer.

Acknowledgments

Received 12/22/2004; revised 3/22/2005; accepted 4/15/2005.

Grant support: SRC grant from the Korea Science and Engineering Foundation and the Non-Directed Research Fund (KRF-99-019-F00063) from the Korea Research Foundation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Michael L. Aitchison, Chang W. Song, Robert J. Griffin, Donatella Del Bufalo, and Yi Sun for their critical reading and invaluable comments on the manuscript. We also thank Haesook Lee and You-Ran Ahn for their assistance with the immunocytochemistry.

References

Promotion of Invasion and Metastatic Potential by Bcl-2

In the article on the promotion of invasion and metastatic potential by Bcl-2 in the July 1, 2005 issue of Cancer Research (1), Dr. Seung Bae Rho was mistakenly omitted from the list of authors. The list of authors should have read: Jihyung Choi, Kyusam Choi, Etty N. Benveniste, Seung Bae Rho, Young-Sook Hong, Je-Ho Lee, Jhingook Kim, and Kyoungsook Park. Also, Dr. Kim should have been listed as a co-corresponding author: Jhingook Kim, Department of Thoracic Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Kangnam-Ku, 135-710, Seoul, Korea. Fax: 82-2-3410-0089; E-mail: jhingook.kim@samsung.com.


©2006 American Association for Cancer Research. doi:10.1158/0008-5472.CAN-66-2-COR
Bcl-2 Promotes Invasion and Lung Metastasis by Inducing Matrix Metalloproteinase-2

Jihyung Choi, Kyusam Choi, Etty N. Benveniste, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/13/5554

Cited articles
This article cites 33 articles, 16 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/13/5554.full#ref-list-1

Citing articles
This article has been cited by 10 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/65/13/5554.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.