The relationship between cell transformation and p38 MAP kinase, a major mitogen-activated protein (MAP) kinase pathway converting signals of various extracellular stimuli into expression of specific target genes through activation of transcription factors, still remains unclear. The aim of the present study was to investigate the role of the p38 MAP kinase pathway in epidermal growth factor (EGF)-induced cell transformation in JB6 cells. Our data show that a dominant negative mutant of p38 MAP (DN-p38) kinase inhibits EGF-promoted JB6 Cl41 cell transformation and that SB203580, an inhibitor of p38 MAP kinase, also inhibits JB6 Cl41 cell transformation in a dose-dependent manner. Moreover, our results show that DN-p38 MAP kinase inhibits the phosphorylation of EGF-stimulated activating transcription factor-2 (ATF-2) and signal transducer and activator of transcription 1 (STAT1). Additionally, DN-p38 MAP kinase inhibits EGF-induced phosphorylation of c-Myc (Thr58/Ser62). Gel shift assays indicate that DN-p38 MAP kinase inhibits EGF-induced activator protein-1 (AP-1) DNA binding in a dose-dependent manner. These results show that p38 MAP kinase plays a key role in the regulation of EGF-induced cell transformation in JB6 cells through regulation of phosphorylation of p38 MAP kinase and activation of its target genes in phosphorylation, c-Myc cell transformation-related genes, and AP-1 binding ability.

The mitogen-activated protein kinases (MAPKs), including p38 kinase, c-Jun NH2-terminal kinases (JNKs), extracellular signal-regulated kinases (ERKs), and signal transduction cascades, are vital mediators of many cellular functions such as growth, development, proliferation, differentiation, malignant transformation, inflammation, and apoptosis (1-3). p38 MAP kinase is activated by cellular stresses including inflammatory cytokines, ultraviolet light, and growth factors, and the activated p38 MAP kinase has been shown to phosphorylate several transcription factors including ATF-2, STAT1, the Max/Mycc complex, and myocyte enhancer factor 2 (MEF2) (1, 4-6). On the other hand, ERKs are predominantly activated by mitogenic stimuli, including mitogens and growth factors, and activated ERKs are involved in cell differentiation and development (2, 3). Although one major function of the p38 kinase and JNKs pathways is regulation of inflammation and apoptosis, in many cases the biological consequences of p38 kinase and JNK activation overlap with those of ERKs in mediation of cell growth and differentiation (2, 3, 6, 7).

Previously, we studied ERKs regulation of epidermal growth factor (EGF)-induced cell transformation in promotion sensitive (P') derivatives of the mouse epidermal JB6 cell line (8). Results showed that inhibition of ERKs appeared to be an important contributor to the tumor promotion-resistant phenotype in JB6 cells. A recent study showed that the p38 kinase and JNKs pathways cooperate to transactivate the vitamin D receptor through the c-Jun/activator protein-1 (AP-1) (9). This result indicates that a connection exists between p38 MAP kinase and AP-1. But AP-1 activation is involved in JB6 cell transformation promoted by EGF and the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (10, 11). AP-1 is a dimeric complex consisting of proteins encoded by the jun and fos gene families, and the AP-1 binding region in these genes is referred to as the TPA response element (TRE). AP-1 induces the transcription of genes that are related to cell proliferation, metastasis, and metabolism (12-14). Increased AP-1 activity is associated with malignant transformation, and repression of AP-1 activity has been shown to lead to suppression of cell transformation and tumor promotion (15).

Activation of MAP kinase cascades is known to play a considerable role in malignant transformation (3, 8). However, whether p38 MAP kinase is involved in the regulation of cell transformation is not known. A recent study showed that only p38 MAP kinase, a MAP kinase usually associated with stress responses, growth arrest, and apoptosis, was consistently increased in human non-small cell lung cancer samples compared with the normal tissues examined (16). More interesting are the results showing that ERKs and JNKs, the MAP kinase pathways traditionally associated with cell growth and perhaps malignant transformation, were not activated in the human non-small cell lung tumor samples. These results indicate that p38 MAP kinase is probably involved in malignant cell transformation. The purpose of this research project was to explore the mechanism of p38-mediated JB6 cell transformation using CMV-neo plasmid-transfected JB6 cells and dominant negative mutant p38 (DN-p38) plasmid-transfected JB6 cells following EGF stimulation. The results obtained show that p38 MAP kinase plays a critical role in regulation of JB6 Cl41 cell transformation promoted by EGF through the inhibition of the phosphorylation of ATF-2, STAT1, and c-Myc and the repression of AP-1 binding ability.
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EXPERIMENTAL PROCEDURES

Materials—Eagle’s minimal essential medium (MEM), t-glutamine, and LipofectAMINE™ were from Invitrogen; EGF and Shionogi’s were from Calbiochem-Novabiochem. Fetal bovine serum (FBS) was from Gemini Bio-Product (Calabasas, CA). Penicillin, streptomycin, and gentamicin sulfate were from BioWhittaker, Inc. (Walkersville, MD). Folin & Ciocalteu’s phenol reagent was from Pierce, and polyvinylidene difluoride (PVDF) membrane was from Millipore (Bedford, MA). Antibodies to detect the phosphorylation of p38 kinase, ERKs, p38, JNKs, ATF-2, STAT1, and c-Myc were used as non-phosphorylated levels of p38 kinase, ERKs, JNKs, ATF-2, STAT1 and the c-Myc protein were from Upstate Biotechnology (Lake Placid, NY). [32P]ATP was from Amersham Biosciences.

Cell Culture and Establishment of Stably Transfected JB6 Cells—Using the CV-MO-neo plasmid and DN-p38 kinase plasmid, we established stable transfectants according to the protocol from Invitrogen. All of these cells were selected in media containing 400 μg/ml of G-418 for 2 weeks, and then the G418 concentration was decreased to 200 μg/ml and maintained. Empty vector CV-MO-neo plasmid-transfected JB6 cells (Cl41 CV-MO-neo) and DN-p38-plasmid-transfected JB6 cells (Cl41 DN-p38) were cultured as adherent monolayers in MEM supplemented with 5% (v/v) heat-inactivated FBS and glutamine (2 m M) at 37°C in a humidified atmosphere of 5% CO₂.

Immunoblotting—Cl41 CMV-neo and Cl41 DN-p38 cells were cultured as described above, and immunoblotting was carried out as described previously (17). In brief, cells were cultured to 80% confluence and then starved in 0.1% FBS/MEM for 24 h at 37°C in a 5% CO₂ incubator. The media were changed to fresh 0.1% FBS/MEM, the cells were washed twice, and then treated with EGF for different time periods at the concentrations indicated. Cells were then treated with lysis buffer (62.5 mM Tris-HCl, pH 6.8, 50 mM dithiothreitol, 2% (v/v) SDS, 10% (v/v) glycerol, and 0.1% bromphenol blue). The lysed samples were transferred into fresh 1.5 ml tubes and sonicated for 5–10 s. Samples containing an equal amount of protein were loaded into each lane of an SDS-polyacrylamide gel for electrophoresis and subsequently transferred onto a polyvinylidene difluoride membrane. Phosphorylation of ERKs, JNKs, ATF-2, STAT1, and c-Myc were selectively detected by Western immunoblotting using a chemiluminescent detection system and a phospho-specific antibody against phosphorylation of p38 kinase (Thr180/Tyr182), ERKs (Thr 202/Tyr204), JNKs (Thr183/Tyr185), ATF-2 (Thr71), STAT1 (Ser727), and c-Myc (Thr58/Ser62). Antibodies against non-phosphorylated levels of p38 kinase, ERKs, JNKs, ATF-2, STAT1 and the c-Myc protein were used as internal controls to determine loading efficiency.

p38 MAP Kinase Activity Assay—A p38 MAP kinase activity assay was carried out following the instructions from Cell Signaling Technol-
SB202190 can specifically inhibit EGF-induced p38 kinase phosphorylation.

**The p38 Kinase Inhibitor SB202190 Suppresses JB6 Cl41 Cell Anchorage-independent Transformation Promoted by EGF**—To confirm the role of p38 MAP kinase in JB6 cell transformation, we explored EGF-promoted cell transformation in JB6 Cl41 cells using the p38 kinase inhibitor SB202190. JB6 cells were treated with EGF (10 ng/ml) alone or with SB202190 (0.1–0.5 μM) in a soft agar matrix at 37 °C in a 5% CO₂ atmosphere for 24 h. Cells were then incubated in fresh 0.1% FBS/MEM for another 2 h before being treated with EGF (10 ng/ml) for 5–60 min or EGF (5–20 ng/ml) for 30 min. Phosphorylation of p38 kinase (C and D), ERKs (A and G), and JNKs (E and K) were determined by Western blot analysis as described under “Experimental Procedures” using specific antibodies against phosphorylation of p38 kinase (Thr180/Tyr182), ERKs and JNKs. Total protein levels of p38 kinase (D and J), ERKs (B and H), and JNKs (F and L) were determined as described under “Experimental Procedures” using corresponding antibodies against non-phosphorylated proteins.

SB202190 can specifically inhibit EGF-induced p38 kinase phosphorylation.

**DN-p38 MAP Kinase Inhibits Phosphorylation of p38 Kinase but Not ERKs or JNKs**—Because SB202190 may affect other molecular targets besides p38 kinase, we used stable transfec-

tants of Cl41 CMV-neo cells and Cl41 DN-p38 cells to further study the biological activity of p38 kinase (24, 25). Cells were treated with EGF at various concentrations for different times,
and Western blot analysis was used to determine the level of phosphorylation of p38 kinase, ERKs, and JNKs using specific antibodies. Our data showed that EGF induced phosphorylation of p38 kinase and ERKs in a dose- (Fig. 4, A and C) and time-dependent (Fig. 4, G and I) manner but had no effect on phosphorylation of JNKs (Fig. 4, E and K). Total protein levels of p38 kinase (B and H), ERKs (D and J), and JNKs (F and L) were determined as described under "Experimental Procedures" using corresponding antibodies against non-phosphorylated proteins.

Fig. 4. DN-p38 MAP kinase inhibits phosphorylation of p38 kinase but not ERKs or JNKs. Empty vector CMV-neo plasmid-transfected JB6 Cl41 cells (Cl41 CMV-neo) and dominant negative p38 plasmid-transfected JB6 Cl41 cells (Cl41 DN-p38) were starved in 0.1% FBS/MEM at 37 °C in a 5% CO₂ atmosphere for 24 h. Cells were then incubated in fresh 0.1% FBS/MEM for another 2 h before being treated with EGF (10 ng/ml) for 15–60 min or EGF (5–20 ng/ml) for 30 min. Phosphorylation of p38 kinase (A and G), ERKs (C and I), and JNKs (E and K) was determined by Western blot analysis as described under "Experimental Procedures" using specific antibodies against phosphorylation of p38 kinase (Thr180/Tyr182), ERKs, and JNKs. Total protein levels of p38 kinase (B and H), ERKs (D and J), and JNKs (F and L) were determined as described under "Experimental Procedures" using corresponding antibodies against non-phosphorylated proteins.

DN-p38 Inhibits Anchorage-independent Transformation Promoted by EGF—From the results described above, we hypothesized that p38 kinase could have a role in regulating JB6 cell transformation. Cell transformation was assessed using our previously developed methods (26, 27). Cl41 CMV-neo cells and Cl41 DN-p38 cells were treated separately with EGF (5–10 ng/ml) in a soft agar matrix at 37 °C in a 5% CO₂ incubator for 10 days. The number of colonies formed was counted automatically by computer, and the same experiment was repeated three times. Our results showed that DN-p38 strongly inhibited the formation of EGF-induced colonies (Fig. 5, D) compared with control cells (Fig. 5, B). The inhibition was evident not only in colony number (Fig. 5, E) but also in colony size (Fig. 5, B versus D). However, untreated Cl41 CMV-neo cells and Cl41 DN-p38 cells showed no colony formation (Fig. 5, A and C). These data indicate that p38 MAP kinase is involved in JB6 cell transformation as a positive regulator rather than an inhibitor.

DN-p38 Inhibits EGF-induced p38 MAP Kinase Activity—To further confirm that the Cl41 DN-p38 stable transfectant cells work as expected, p38 MAPK activity toward its substrate, ATF-2, was assayed. Cells were treated with EGF (10 ng/ml) for the indicated times, and the cell extracts were incubated with an immobilized p38 kinase antibody. An ATF-2 fusion protein was used as a substrate of p38 MAP kinase, and phosphorylation of ATF-2 at Thr71 was detected by Western blot. Our results showed that EGF-induced phosphorylation of ATF-2 at Thr71 was decreased distinctly in Cl41 DN-p38 cells compared with Cl41 CMV-neo cells (Fig. 6). These data indicate that p38 kinase has a role in EGF-induced phosphorylation of ATF-2.
DN-p38 Inhibits EGF-induced Phosphorylation of STAT1 at Ser^{727} in a Time- and Dose-dependent Manner—STAT1 is downstream of p38 MAP kinase and is a transcription factor that mediates cytokine and growth factor-induced signals that culminate in various biological responses, including proliferation and differentiation (28). Cl41 DN-p38 cells and Cl41 CMV-neo cells were treated separately for various times and at various doses with EGF. We found that EGF (10 ng/ml) strongly induced phosphorylation of STAT1 at Ser^{727} in Cl41 CMV-neo cells, but the phosphorylation of STAT1 at Ser^{727} in Cl41 DN-p38 cells was relatively weak. However, phosphorylation of STAT1 gradually decreased within 120 min in both cell lines (Fig. 7A). Non-phosphorylated levels of STAT1 were unchanged throughout the time course and dose course (Fig. 7, B and D). EGF-induced phosphorylation of STAT1 at Ser^{727} gradually increased with increasing EGF concentrations (Fig. 7C) with no change in non-phosphorylated levels of STAT1 (Fig. 7D).

DN-p38 Inhibits Phosphorylation of c-Myc at Thr^{58}/Ser^{62}—To understand the mechanism of p38 MAP kinase in the regulation of JB6 cell transformation, we investigated the phosphorylation of c-Myc by Western blot using a specific antibody against phosphorylation of c-Myc at Thr^{58}/Ser^{62}. The c-Myc oncoprotein is associated with cell growth, development, and malignant transformation in human tumors and cell lines (29, 30). The active c-Myc also contributes to cell transforming potencies in rat embryo cells (31). In this study, our results showed that phosphorylation of c-Myc at Thr^{58}/Ser^{62} was increased after CMV-neo JB6 cells were treated from 1–4 h with EGF (10 ng/ml), but phosphorylation of c-Myc at Thr^{58}/Ser^{62} in DN-p38-JB6 cells was relatively less within the same time course (Fig. 8A). EGF had no effect on total c-Myc protein levels in Cl41 CMV-neo cells or Cl41 DN-p38 cells (Fig. 8, B and D). Moreover, we studied the effects of various doses of EGF on phosphorylation of c-Myc at Thr^{58}/Ser^{62}. Results showed that phosphorylation of c-Myc at Thr^{58}/Ser^{62} increased in Cl41 CMV-neo cells but was only slightly changed in Cl41 DN-p38 cells after cells were treated with EGF (5–20 ng/ml) (Fig. 8C). These data indicate that phosphorylation of c-Myc at Thr^{58}/Ser^{62} is induced by EGF in a time- and dose-dependent manner in Cl41 CMV-neo cells and that DN-p38 inhibits the phosphorylation of c-Myc at Thr^{58}/Ser^{62}.

A Mutant of STAT1 (S727A) Suppresses JB6 Cl41 Cell Anchorage-independent Transformation Promoted by EGF—Stable transfectant JB6 Cl41-STAT1 (S727A) cells expressing STAT1 (S727A) and JB6 Cl41-pcDNA3.1 cells expressing plasmid pcDNA3.1 cells were employed to detect the cell transformation ability promoted by EGF. Cells were treated with EGF (10 ng/ml) in a soft agar matrix at 37 °C in a 5% CO_{2} incubator for 10 days. The colony number was determined as described previously (8). The colony numbers were greatly decreased in JB6 Cl41-STAT1 (S727A) cells stimulated by EGF, but there were many more colonies in JB6 Cl41 cells and JB6 Cl41-pcDNA3.1 stimulated by EGF under the same condition (Fig. 9, A–F). These data indicate that STAT1, one of the targets of p38 MAP kinase, also contributes to JB6 Cl41 cell transformation promoted by EGF.

DN-p38 MAP kinase inhibits p38 MAP kinase activity in vitro. Cl41 CMV-neo cells and Cl41 DN-p38 cells were seeded in 100-mm dishes with 5% FBS/MEM and incubated at 37 °C in a 5% CO_{2} atmosphere for 24 h. Cells were starved as described previously. Cells were then incubated in fresh 0.1% FBS/MEM for another 2 h before being treated with EGF (10 ng/ml) for the indicated time periods. Cells were added to lysis buffer as described under “Experimental Procedures,” and the same amount of extracted proteins was immunoprecipitated with a monoclonal phospho-specific antibody against p38 MAP kinase (Thr^{180/Tyr^{182}}). The resulting immunoprecipitate was incubated with an ATP-2 fusion protein in the presence of ATP and kinase buffer. Phosphorylation of ATP-2 at Thr^{182} was measured by Western blotting using the phospho-ATP-2 (Thr^{182}) antibody (A), and total ATP-2 protein level was determined by Western blot using an antibody against non-phosphorylated ATP-2 protein (B).

DN-p38 MAP kinase inhibits phosphorylation of STAT1 (Ser^{727}). Culture and starvation of Cl41 CMV-neo cells and Cl41 DN-p38 cells were performed as described above. In the time course study (A and B), cells were treated with EGF (10 ng/ml) for the indicated time periods and then harvested with lysis buffer, and the protein concentration of each sample was determined. Equal amounts of protein were separated by 8% SDS-PAGE gel and analyzed by Western blotting. Phosphorylation of STAT1 at Ser^{727} was detected using a specific phospho-STAT1 (Ser^{727}) antibody (A). Total STAT1 protein levels were detected by a non-phospho-STAT1 antibody (B). In the dose course study (C and D), Cl41 CMV-neo cells, and Cl41 DN-p38 cells were treated with EGF at the indicated concentration for 15 min. Phosphorylation of STAT1 at Ser^{727} (C) and total STAT1 protein levels (D) were detected as described above.
DN-p38 Inhibits EGF-induced AP-1 Binding Activity—AP-1 induces gene transcription by binding to the TPA response element site in the promoter region of its target gene and is required for cell proliferation, differentiation, and malignant transformation (32, 33). Double-stranded AP-1 oligonucleotides were labeled with $^{32}$P, and electrophoretic mobility-shift assays were used to measured AP-1 DNA binding activity. Our results showed that AP-1 binding was decreased in Cl41 DN-p38 cells (Fig. 10, A and B, lanes 3, 4, and 5) compared with EGF-induced AP-1 DNA binding in Cl41 CMV-neo cells (lanes 7–9). However, no significant change was observed between DN-p38-JB6 cells and Neo-JB6 cells without EGF stimulation (Fig. 10, A and B, lanes 2 and 6). To confirm that the electrophoretic mobility-shift band was specific for AP-1 binding, a 10-fold amount of unlabeled AP-1 oligonucleotide was added, and results showed that the AP-1 binding band was completely removed (Fig. 10, A and B, lane 1). These data indicate that DN-p38 inhibits EGF-induced AP-1 binding ability.

**DISCUSSION**

Previous studies show that ERKs are involved in the JB6 cell transformation promoted by EGF or TPA (8), but an understanding of the role of p38 MAP kinase in cell transformation still remains unclear. In this study, we found that p38 MAP kinase plays a critical role in JB6 Cl41 cell transformation promoted by EGF. Both DN-p38 and the p38 MAP kinase inhibitor SB202190 suppressed JB6 Cl41 cell transformation promoted by EGF. DN-p38 inhibited EGF-stimulated phosphorylation of ATF-2, STAT1, and c-Myc, and gel shift assay results showed that DN-p38 inhibits AP-1 binding ability. These results indicate that p38 MAP kinase is involved in regulation of JB6 Cl41 cell transformation promoted by EGF through the inhibition of phosphorylation of its downstream factors, including ATF-2, STAT1, c-Myc, and also AP-1 DNA binding.

Many studies indicate that EGF induces the phosphorylation of ERKs and p38 MAP kinase (21, 34–36). The ERK pathway is associated with activation of the EGF receptor and has been shown to play a major role in promoting several tumor phenotypes. However, the JNKs pathway has not been shown to be activated through the EGF receptor but is instead more uniformly stimulated by cellular stresses and cytokines (21). Our present data also show that EGF only induces the phosphorylation of ERKs and p38 MAP kinase but not JNKs (Fig. 1).
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SB202190 and DN-p38 only inhibit EGF-induced phosphorylation of p38 MAP kinase but not ERKs (Figs. 2 and 4). These results indicate that ERKs and p38 MAP kinase, but not JNKs, independently participate in EGF-stimulated cell signal transduction.

The p38 MAP kinase is usually associated with stress responses, growth arrest, and apoptosis (1, 2, 4). But a recent study (16) showed that it was activated in human lung cancer samples, suggesting an additional role for this pathway in malignant cell growth or transformation. The present study is the first to report that p38 MAP kinase is involved in JB6 Cl41 cell transformation promoted by EGF. SB202190, a p38 MAP kinase inhibitor, distinctly inhibited colony formation in EGF-promoted JB6 Cl41 cell transformation (Fig. 3). To eliminate the effects of SB202190 on other molecular targets, we successfully constructed a stable transfectant of JB6 Cl41 cells expressing a dominant negative mutant of p38 kinase and DN-p38 according to the method described previously (37, 38). Our results showed that the number of colonies in EGF-treated Cl41 DN-p38 cells was significantly decreased compared with the number of colonies in Cl41 CMV-neo cells (Fig. 5). These data indicate that p38 MAP kinase plays a critical role in JB6 Cl41 cell transformation promoted by EGF.

To explore the mechanism of p38 kinase in the regulation of JB6 cell transformation, we first determined whether p38 MAP kinase activity was inhibited in Cl41 DN-p38 cells. Our results show that DN-p38 distinctly inhibits EGF-induced phosphorylation of ATF-2 at Thr71 (Fig. 6). Thr71 is a major ATF-2 phosphorylation site required for transcriptional activity, and a high level of phosphorylated ATF-2 protein correlates with malignant phenotypes in the multistage mouse skin carcinogenesis model (39). One recent study showed that EGF activates transcription factor ATF-2 through a two-step mechanism. The Raf-MEK-ERK pathway induces phosphorylation of ATF-2 (Thr71), whereas subsequent ATF-2 Thr69 phosphorylation requires the Raf-RalGDS-Src-p38 MAP kinase pathway (40). Moreover, cooperation between ERKs and p38 kinase was found to be essential for ATF-2 activation by EGF (41).

Some of the STAT family members have a role in the regulation of cellular transformation (42). A recent study showed that STAT3 activation is required for interleukin-6-induced transformation in tumor promotion-sensitive mouse skin epithelial cells (43). Another report showed that transformation of normal fibroblasts cells with E1A + Ha-Ras oncogenes causes a constitutive activation of STAT1 and STAT3 transcription factors (44). In this study, we found that EGF-induced phosphorylation of STAT1 (Ser727) also decreased in Cl41 DN-p38 cells but increased in Cl41 CMV-neo cells after the cells were separately treated with EGF (10 ng/ml) (Fig. 7). We successfully employed directed point mutation technology to obtain a mutant of STAT1 at Ser727 (Ser → Ala). Stable transfectant of JB6 Cl41-STAT1 (S727A) cells expressing mutant of STAT1 at Ser727 (Ser → Ala) displayed its ability to inhibit cell transformation in JB6 Cl41 cells promoted by EGF (Fig. 9). These results indicate that EGF-induced phosphorylation of STAT1 at Ser727 is at least partially dependent on p38 MAP kinase and that STAT1 is involved in JB6 cell transformation promoted by EGF.

Studies show that the c-Myc oncoprotein is associated with cancer cell proliferation and transformation (45, 46). In this study, we also found that DN-p38 inhibited EGF-induced phosphorylation of c-Myc, indicating that p38 MAP kinase mediates EGF-induced c-Myc phosphorylation (Fig. 8). Thus, p38 MAP kinase may be involved in cell transformation through phosphorylation of c-Myc. On the other hand, AP-1, a heterodimer commonly comprised of the basic leucine zipper proteins Fos and Jun, plays a very important role in the induction of neoplastic transformation and the multiple genes involved in cell proliferation, differentiation, and inflammation (47, 48). Blocking the tumor promoter-induced activation of AP-1 was shown to inhibit neoplastic transformation in JB6 mouse epidermal cells (49). One recent study (47) showed that p38 MAPK and ERK inhibition with SB203580 and PD98059, respectively, significantly inhibited silica-induced AP-1 activation. These findings demonstrate that AP-1 activation may be mediated through the p38 MAPK and ERKs pathways (47). In the present research, EGF-induced AP-1 binding ability decreased in Cl41 DN-p38 cells compared with Cl41 CMV-neo cells (Fig. 10). These data provide further evidence that EGF-induced AP-1 binding ability is mediated through p38 MAP kinase and that DN-p38 MAP kinase represses EGF-induced JB6 Cl41 cell transformation through inhibition of AP-1 DNA binding ability.

In summary, we report for the first time that p38 MAP

![Proposed signal transduction pathways of EGF-promoted JB6 Cl41 cell transformation](http://www.jbc.org/content/journal/jbc/264/11/32621F11.large.jpg)

**Fig. 11.** Proposed signal transduction pathways of EGF-promoted JB6 Cl41 cell transformation. We reported previously that ERKs are necessary factors in EGF-promoted JB6 Cl41 cell transformation. In the present study, our data show that DN-p38 MAP kinase effectively blocks EGF-promoted JB6 Cl41 cell transformation through inhibition of the phosphorylation of transcription factors, ATF-2 and STAT1, oncogenes, c-Myc, and repression of AP-1 DNA binding. Both p38 kinase and ERKs are involved in the EGF-promoted JB6 Cl41 cell transformation.
kinase mediates EGF-promoted JB6 Cl41 cell transformation through regulation of the phosphorylation of its downstream factors, such as STAT1, ATF-2, and c-Myc, and also AP-1 DNA binding ability (Fig. 11). This study illustrates that p38 MAP kinase is another new pathway involved in JB6 Cl41 cell transformation promoted by EGF.

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