Activation of Fibroblast Collagenase-1 Expression by Tumor Cells of Squamous Cell Carcinomas Is Mediated by p38 Mitogen-activated Protein Kinase and c-Jun NH₂-terminal Kinase-2

Jukka Westermarck, Songping Li, Panu Jaakkola, Tuula Kallunki, Reidar Grénman, and Veli-Matti Kähäri

Turku Centre for Biotechnology, University of Turku and Åbo Akademi University [J. W., S. L., P. J., V.-M. K.], MediCity Research Laboratory [J. W.] and Department of Medical Biochemistry [V.-M. K.], University of Turku, and Department of Dermatology [V.-M. K.], and Otorhinolaryngology-Head and Neck Surgery [R. G.], Turku University Central Hospital, FIN-20520 Turku, Finland, and Apoptosis Laboratory, Institute of Cancer Biology, Danish Cancer Society, DK-2100 Copenhagen, Denmark [T. K.]

ABSTRACT

Collagenase-1 (matrix metalloproteinase [MMP]-1) is one of the few proteinases capable of degrading fibrillar collagens, and it is expressed by several types of normal and malignant cells (1, 2). In addition, enhanced expression of MMP-1 has been shown to correlate with poor prognosis of several types of malignant tumors (4–6). The expression of MMP-1 in invasive neoplastic tumors, e.g., SCCs, of the head and neck and vulva is detected primarily in the stromal compartment (7, 8), suggesting, that the expression of MMP-1 in peritumoral fibroblasts is induced in paracrine manner by tumor cells and tumor-infiltrating inflammatory cells. A single AP-1 element located at −65 to −72 in the promoter region of human MMP-1 gene plays a critical role in the activation of MMP-1 gene transcription in response to a variety of extracellular signals (9). In addition, other regulatory cis-elements, including the ETS element adjacent to AP-1 binding site, play a role in the regulation of AP-1-dependent MMP-1 gene transcription (10, 11).

MAPK signaling modules mediate the induction of the expression and the activity of AP-1 and ETS transcription factors in response to extracellular stimuli (12, 13). At present, three distinct MAPK pathways are known in detail: (a) ERK1/2 (b) JNK/SAPK; and (c) p38 MAPK. The ERK1/2 pathway (Raf→MEK1/2→ERK1/2) is activated by mitogenic growth factors via Ras and by phorbol esters via protein kinase C. In contrast, the stress-activated MAPK pathways JNK/SAPK (MEK kinase 1–3→MAPK kinase 4 and 7→JNK/SAPK) and p38 (MAPK kinase kinase 3 and 6→p38) are activated by cellular stress, e.g., UV light and osmotic and oxidative stress, and by inflammatory cytokines. The ERK1/2 pathway has been shown to mediate activation of minimal MMP-1 promoter by serum, phorbol ester, insulin, and oncostatin M (14–16). Activity of p38 MAPK is required for IL-1-elicited induction of MMP-1 expression in fibroblasts and endothelial cells (17), whereas enhancement of fibroblast MMP-1 expression by lipid second messenger ceramide and tumor promoter okadaic acid involves coordinate activation of the ERK1/2, JNK/SAPK, and p38 MAPK pathways (18, 19).

In this study, we have examined the role of MAPK pathways in regulation of fibroblast MMP-1 expression using an experimental approach that would mimic the environment of peritumoral fibroblasts in the invasive SCCs of the head and neck. We show that SCC tumor cell-derived soluble factors activate fibroblast MMP-1 expression at the transcriptional level and that this is mediated by p38 MAPK. In addition, tumor cell-elicited induction of collagenase-3 expression in murine fibroblasts is dependent on activation of JNK2. These results show for the first time that stress-activated MAPK pathways play an important role in tumor cell-induced activation of collagenolytic phenotype of fibroblasts and identify the JNK2 pathway as a novel therapeutic target for inhibition of SCC invasion.

MATERIALS AND METHODS

Reagents and Antibodies. Recombinant TNF-α, IL-1β, TGF-α, GM-CSF, HB-EGF, EGF, and TGF-β were obtained from Sigma. Blocking antibodies against TNF-α and IL-1β were obtained from Genzyme. MEK1/2 inhibitor PD98059, p38 inhibitor SB203580, and PI3K inhibitor LY294002 were purchased from Calbiochem (San Diego, CA). Phosphospecific ERK1/2, JNK,
RESULTS

Induction of Fibroblast MMP-1 Expression by SCC Tumor Cell Media. To study the regulation of fibroblast MMP-1 gene expression by SCC tumor cells, we collected conditioned media (TM) of six low-passage tumor cell lines established from SCCs of the oral cavity and larynx and used them to treat SCC stromal fibroblasts in culture. Initially, fibroblasts were incubated with medium containing 30% or 50% of SCC TM, and their MMP-1 mRNA expression was studied by Northern blot hybridizations. As shown in Fig. 1A, conditioned media of tumor cell lines UT-SCC-2, UT-SCC-14, and UT-SCC-18 (called +TM below) potently enhanced MMP-1 mRNA expression, whereas treatment with conditioned media of tumor cell lines UT-SCC-6A, UT-SCC-6B, and UT-SCC-8 (called −TM below) did not markedly alter MMP-1 mRNA levels in fibroblasts. Nearly maximal activation of fibroblast MMP-1 mRNA expression was obtained with 30% of +TM, and this concentration was therefore used in additional experiments. Similar results were also obtained with another SCC tumor fibroblast line and normal human skin fibroblasts incubated with same media (data not shown).

Next, stromal fibroblasts were treated simultaneously with +TM and −TM in different ratios to rule out the possibility that inability of −TM to activate fibroblast MMP-1 expression was due to the presence of inhibitory factor(s) that could block activation of MMP-1 expression by stimulatory factor(s) in the same media. Interestingly, treatment with a high concentration (70% or 90%) of −TM (of UT-SCC-6A) also enhanced fibroblast MMP-1 expression, and a mixture of +TM (10%) and −TM (90%) activated MMP-1 expression 3.3-fold more potently than 10% +TM (of UT-SCC-2) alone (Fig. 1B). Furthermore, total protein concentration of tumor media did not correlate with the capacity to activate MMP-1 expression (data not shown), suggesting that the difference between −TM and +TM is based on the difference in the concentration of stimulatory factor(s) in the media.

Next, we compared the level of induction of fibroblast MMP-1 mRNA expression by 30% +TM to that obtained by treatment with TNF-α, IL-1β, TGF-α, EGF, HB-EGF, and GM-CSF. As shown in Fig. 2A, IL-1β and TNF-α stimulate MMP-1 mRNA expression nearly as potently as +TM, whereas EGF, HB-EGF, and TGF-α were clearly less potent in enhancing MMP-1 mRNA abundance in fibroblasts, and GM-CSF had no marked effect. To study, whether the inducing factor in the +TM would be TNF-α or IL-1β, we treated tumor fibroblasts with +TM

![Fig. 1. Induction of stromal fibroblast MMP-1 expression by SCC tumor cells. A. human head and neck SCC stromal fibroblasts were treated for 24 h with medium containing 30% or 50% of conditioned medium of different human SCC tumor cell lines, as indicated. B. SCC stromal fibroblasts were treated for 24 h with different ratios of conditioned media of SCC tumor cell lines, as indicated.](cancersres.aacrjournals.org)
neously with +TM had no effect on the induction of fibroblast MMP-1 expression by +TM (Fig. 2C, Lanes 6 and 7).

**Activation of MMP-1 Gene Transcription by SCC Tumor Cell Media.** We and others have shown that the MMP-1 gene promoter segment at −95 to −65, which contains the adjacent AP-1 and ETS binding sites, plays an important role in the activation of MMP-1 gene transcription in fibroblasts (10, 11). To examine the regulation of MMP-1 gene promoter activity by SCC TM, we transiently transfected neonatal human foreskin fibroblasts with 5′ deletion constructs of the human MMP-1 promoter linked to CAT reporter gene and measured CAT activity as an indicator of promoter activity after a 36-h treatment of fibroblasts with +TM. As shown in Fig. 3A, the activity of −55CLCAT, which contains the basal promoter region of the MMP-1 gene, was only slightly (1.3-fold) enhanced by treatment of fibroblasts with +TM, whereas the activity of −72CLCAT, which contains the AP-1 element, was stimulated 4.4-fold. Interestingly, the presence of the ETS element in −95CLCAT or other upstream elements in −2278CLCAT did not increase MMP-1 promoter activation by +TM treatment (Fig. 3A), providing evidence that the AP-1 site plays a major role in stimulation of MMP-1 promoter activity in response to SCC TM.

To further study the role of AP-1 and ETS cis-elements in tumor cell-elicited activation of the MMP-1 promoter, we performed *in vitro* DNase footprinting using a MMP-1 promoter fragment extending from −60 to −115 as a probe. Interestingly, the AP-1 element at −72 to −65 was clearly protected with nuclear proteins from untreated control fibroblasts, and no alteration in protection was observed after treatment with +TM for 6, 8, and 12 h (Fig. 3B; data not shown), indicating that the activation of MMP-1 promoter by +TM is not associated with increased occupancy of the AP-1 binding site. In accordance with the data above, the ETS element at position −89 to −83 was not protected by nuclear proteins from control or +TM-treated fibroblasts (Fig. 3B). These results indicate that the activation of MMP-1 gene expression by +TM occurs at the promoter level and suggest that it is mediated through activation of prebound AP-1 transcription factors but not through the ETS element.

**Regulation of AP-1 Expression and Activity by Tumor Cell Media.** To study the regulation of AP-1 expression by tumor media, we treated stromal fibroblasts with both +TM and −TM for different periods of time and determined MMP-1 and AP-1 mRNA abundance by Northern blot hybridizations. Interestingly, treatment with both +TM and −TM induced expression of *junB*, c-*fos* (Fig. 4, A and B), and *fra-1* (data not shown) mRNA equally potently, whereas induction of c-*jun* mRNA by +TM declined markedly later on when compared with induction by −TM (Fig. 4, A and B). Neither ets-1 nor ets-2 mRNA was induced by tumor cell media (data not shown).

Activation of c-*jun* transcription is mediated by INK and p38 MAPK, which phosphorylate ATF-2 and c-Jun bound to the AP-1 site in the promoter of c-*jun* (12). To study the activation of c-Jun and ATF-2 by SCC tumor cell media, we treated tumor fibroblasts with +TM and −TM and examined phosphorylation of c-Jun and ATF-2 by Western blot analysis using phosphospecific antibodies. As shown in Fig. 4C, treatment of fibroblasts with +TM induced phosphorylation of c-Jun from 30–120 min, whereas no phosphorylated form of c-Jun was observed in response to −TM treatment. Moreover, ATF-2 phosphorylation was clearly increased at 15 and 30 min of incubation with +TM, as compared with −TM. In contrast, treatment with −TM induced phosphorylation of transcription factor CREB slightly more potently (Fig. 4C). Taken together, these results show that activation of signaling pathways that induce c-Jun and ATF-2 phosphorylation specifically correlates with induction of MMP-1 expression by SCC tumor cell-derived factors and suggest that MMP-1 gene expression is regulated by mechanisms involving posttranscriptional regulation of AP-1 activity.
and B by both phosphospecific antibodies. Interestingly, ERK1/2 was equally activated by both MMP-1 expression by tumor cell media, we first examined the mean of five independent experiments.

Activity. Induction of promoter activity compared with untreated cells (1.00) represents the mean of five independent experiments. TM

UT-SCC-2 (1) or from cells treated with 50% of conditioned medium of SCC tumor cell line (NAKED) alone (Naked) or together with nuclear extracts from untreated control cells (CTC) or from cells treated with 50% of conditioned medium of UT-SCC-2 tumor cells (+TM). The samples were fractionated on an 8% polyacrylamide sequencing gel in parallel with the A + G sequencing ladder of the same promoter fragment. The location of the AP-1 and ETS binding sites is indicated, and the sequence of the elements in antisense orientation is shown. A representative experiment of four independent experiments with identical results is shown.

INDUCTION OF Stromal Fibroblast MMP-1 EXPRESSION

Fig. 3. Activation of MMP-1 promoter by SCC TM. A, human neonatal foreskin fibroblasts were transiently transfected with distinct human MMP-1 promoter/CAT constructs and treated for 36 h with 50% of conditioned medium of SCC tumor cell line UT-SCC-2 (+TM), followed by an assay of CAT activity as an indicator of promoter activity. Induction of promoter activity compared with untreated cells (1.00) represents the mean of five independent experiments. B, DNase I footprinting was performed with end-labeled MMP-1 promoter fragment (−60 to −178) alone (Naked) or together with nuclear extracts from untreated control cells (CTC) or from cells treated with 50% of conditioned medium (TM) or tumor cells (TM). The samples were fractionated on an 8% polyacrylamide sequencing gel in parallel with the A + G sequencing ladder of the same promoter fragment. The location of the AP-1 and ETS binding sites is indicated, and the sequence of the elements in antisense orientation is shown. A representative experiment of four independent experiments with identical results is shown.

Induction of MMP-1 Expression by SCC TM Is Mediated by p38.
To study the role of distinct MAPK pathways in the induction of fibroblast MMP-1 expression by tumor cell media, we first examined the activation of ERK1/2, JNK, and p38 by Western blot analysis using phosphospecific antibodies. Interestingly, ERK1/2 was equally activated by both +TM and −TM in a time range from 15 min to 1 h (Fig. 5, A and B). In contrast, JNK and p38 were clearly more potently activated by +TM (Fig. 5, A and B), suggesting a role for JNK and p38 MAPK in the activation of fibroblast MMP-1 gene expression by SCC tumor cells.

To directly examine the role of distinct MAPK pathways in the tumor medium-elicited activation of MMP-1 expression, we blocked ERK1/2 and p38 MAPK pathways by chemical inhibitors previously shown to block MMP-1 gene activation by diverse stimuli (17–19). As seen in Fig. 6A, activation of fibroblast MMP-1 mRNA abundance by +TM was potently inhibited by SB203580, a specific inhibitor of p38 activity. In contrast, treatment of fibroblasts with PD98059, an inhibitor of ERK1/2 activators MEK1/2, had no marked effect on the induction of MMP-1 mRNA levels by +TM (Fig. 6, A and B), although it potently abrogated induction of MMP-1 mRNA abundance by C2-ceramide (Fig. 6B). In parallel, blocking the activity of PI3k by specific inhibitor LY294002 had no marked effect on the activation of fibroblast MMP-1 expression by +TM (Fig. 6A).

Induction of Mouse Fibroblast Collagenase-3 Expression by SCC TM Is Mediated by JNK2.
To study the role of the JNK pathway in the TM-elicited induction of fibroblast MMP-1 expression, we treated embryonal fibroblasts derived from a JNK2 knockout mouse (JNK2−/−) and from a corresponding wild-type mouse (JNK2+/+) with +TM (of UT-SCC-2 and UT-SCC-14) and determined the expression of murine collagenase-3 (MMP-13), the only murine fibroblast collagenase. Interestingly, +TM treatment clearly induced the expression of mouse collagenase-3 mRNA in JNK2+/+ fibroblasts, but not in JNK2−/− fibroblasts (Fig. 7A). Similar results were obtained with two embryonal fibroblast cell lines from two distinct strains of JNK2+/+ and JNK2−/− mice. To confirm that the lack of mouse collagenase-3 mRNA induction by +TM in JNK2−/− cells is not due to general suppression of gene regulation, we treated JNK2−/− and JNK2+/+ mouse fibroblasts with okadaic acid, which is known to activate MMP-1 gene expression through MAPK signaling (19), and with TGF-β, a down-regulator of fibroblast MMP-1 expression (28). As shown in Fig. 7B, treatment with okadaic acid up-regulated mouse MMP-13 mRNA levels, and treatment with TGF-β down-regulated mouse MMP-13 mRNA levels in both JNK2−/− and JNK2+/+ mouse fibroblasts. The specific role of JNK signaling in tumor medium-induced mouse fibroblast MMP-13 expression was further confirmed by showing that ERK1/2 was similarly activated in both JNK2−/− and JNK2+/+ cells (Fig. 7C).

DISCUSSION
Tumor growth and invasion is a complex process that involves interactions between tumor cells and fibroblasts of the surrounding stromal tissue. Degradation of stromal ECM by MMPs apparently plays an important role in growth and invasion of malignant tumors, and in general, MPPs are produced by tumor cells, peritumoral stromal fibroblasts, and tumor-infiltrating inflammatory cells (1–3). Malignant tumor cells also induce expression of distinct MPPs in peritumoral fibroblasts. For example, breast carcinoma cell-derived IL-1 potently enhances expression of MMP-13 in stromal fibroblasts (29). Interestingly, recent findings also suggest that stromal fibroblasts play a role in regulation of the malignant epithelial cell phenotype and that this may be achieved by fibroblast-derived MPPs (30–32). Previous studies using recombinant growth factors and cytokines and purified tumor promoters have been successful in elucidating signaling mechanisms involved in the regulation of MPP gene expression, but this experimental approach does not take into account the in vivo condition, in which tumor cells and stromal fibroblasts are exposed to a variety of tumor cell-derived factors. In the present study, we show that a subgroup of low-passage primary tumor cell lines established from human head and neck SCCs secretes factor(s) that induce MMP-1 expression in normal and tumor fibroblasts. Although we were not able to identify the collagenolytic phenotype-inducing factor in TM, we show that it is neither TNF-α nor IL-1β and that the effect is not dependent on MMP activity and requires activity of p38 MAPK and JNK2.

7159

Downloaded from cancerres.aacrjournals.org on September 23, 2017. © 2000 American Association for Cancer Research.
Increased AP-1 activity has been shown to transform benign cells and enhance tumor cell invasion and metastasis (33). Although enhanced expression of AP-1 genes in malignant tumors in vivo has been reported, there is no consistent pattern that would serve as a marker for increased invasion or malignancy (34–36). Interestingly, even reduced expression of c-jun, junB, and c-fos genes was observed in human lung carcinomas, as compared with normal tissue (37). In the present study, prolonged activation of fibroblast c-jun expression by SCC tumor cell media is associated with stimulation of MMP-1 gene expression, whereas equal activation of c-fos, junB, and fra-1 mRNA expression by −TM and +TM suggests that these AP-1 genes do not play a role in the induction of fibroblast MMP-1 gene expression. Our results also show that the increased phosphorylation of c-Jun in cells treated with +TM was more pronounced than the difference seen in the regulation of c-jun expression, further suggesting that phosphorylation of AP-1 components plays a key role in the regulation of MMP-1 transcription. Furthermore, induction of c-jun expression by −TM treatment in the absence of c-Jun phosphorylation suggests that activation of c-jun expression may be mediated, in part, by c-Jun phosphorylation-independent mechanisms such as the ERK5–MEF2 pathway (38).

We have recently shown that the activation of fibroblast MMP-1 expression by ceramide and okadaic acid is mediated by coordinate activation of ERK1/2, JNK/SAPK, and p38 MAPK pathways (18, 19). Furthermore, MEK1 activity is required for increased expression of MMP-1 in Ras-transformed human fibroblasts (34). Moreover, it has been shown that constitutive activation of ERK1/2 results in transformation of fibroblasts (39) and that the ERK1/2 pathway is activated in malignant tumors in vivo (40, 41). Interestingly, the results of the present study clearly show that ERK1/2 activation is not required for the induction of fibroblast MMP-1 expression by +TM and that, in the absence of JNK2, activation of the ERK1/2 pathway is not sufficient for induction of murine fibroblast MMP-13 expression. Our results also show that ERK1/2 activation and induction of junB and c-fos mRNA expression are similar with both −TM and +TM, which is in accordance with a recent study showing that the expression of c-fos and junB is regulated primarily by the ERK1/2 pathway (42). Taken together, these results clearly show that, in contrast to other stimuli (18, 19), ERK1/2 activation does not play a role in the paracrine regulation of fibroblast MMP-1 expression by SCC tumor cells. Our results also show that activity of p38 MAPK is critical in induction of fibroblast MMP-1 expression by SCC cell media. These results are in accordance with our previous observations showing the importance of the p38 MAPK pathway in induction of MMP-1 expression in fibroblasts and in SCC cells (18, 19, 43).

Our observations, which suggest that induction of MMP-1 promoter activity in response to +TM treatment takes place without alterations in the occupancy of MMP-1 promoter AP-1 binding element, are in accordance with previous results showing that inhibition of phorbol ester-elicited MMP-1 promoter activation by dexamethasone does not alter AP-1 site occupation (44). Interestingly, it was recently reported that dexamethasone inhibits JNK activity and phosphorylation of c-Jun (45), emphasizing the role of JNK in the activation of prebound AP-1 complexes by phosphorylation, as has been shown previously in the activation of c-jun promoter (46). Because JNK2 displays a higher affinity for c-Jun than other JNK isoforms (47), we assessed the role of JNK signaling in tumor medium-elicited enhancement of collagenolytic phenotype of fibroblasts using embryonal fibroblasts from JNK2 knockout mice. No murine homologue for human MMP-1 has been identified; therefore, we determined the expression of murine collagenase-3 (MMP-13), the only murine fibroblast collagenase in these cells. Murine MMP-13 promoter

---

1. J. Westermarck, S-P. Li, T. Kallunki, J. Han, and V-M. Kahari. p38 MAPK-dependent activation of protein phosphatase-1 and 2A inhibits MEK1,2 activity and collagenase-1 (MMP-1) gene expression, submitted for publication.
Ceramide (fibroblasts were incubated for 24 h with 30% of conditioned medium of SCC tumor cell line UT-SCC-2 or UT-SCC-14 cells (+TM) or with conditioned medium of UT-SCC-8 or UT-SCC-6A cells (~TM) for the indicated periods of time. Thereafter, cells were lysed to sample buffer, and activation of ERK1/2, JNK, and p38 MAPK was determined by Western blot analysis using phosphospecific antibodies for the corresponding MAPKs. Analysis of total p38 was performed as a loading control. Cell lysate of HaCaT keratinocytes treated with EGF (20 ng/ml) for 20 min were used as a positive control. The levels of activated ERK1/2 (p-ERK), JNK (p-JNK), and p38 MAPK (p-p38), as well as total ERK1/2, JNK, and p38, are shown relative to the levels at time point 0 h (1.0).

![Fig. 5. Activation of fibroblast JNK and p38 by SCC TM. Tumor fibroblasts were treated with 30% of conditioned medium of UT-SCC-2 or UT-SCC-14 cells (+TM) or with conditioned medium of UT-SCC-8 or UT-SCC-6A cells (~TM) for the indicated periods of time. Thereafter, cells were lysed to sample buffer, and activation of ERK1/2, JNK, and p38 MAPK was determined by Western blot analysis using phosphospecific antibodies for the corresponding MAPKs. Analysis of total p38 was performed as a loading control. Cell lysate of HaCaT keratinocytes treated with EGF (20 ng/ml) for 20 min were used as a positive control. The levels of activated ERK1/2 (p-ERK), JNK (p-JNK), and p38 MAPK (p-p38), as well as total ERK1/2, JNK, and p38, are shown relative to the levels at time point 0 h (1.0).]

- **Fig. 6.** TM-elicited induction of fibroblast MMP-1 expression is mediated by p38 MAPK. A, tumor fibroblasts were incubated for 24 h with 30% of conditioned medium of SCC tumor cell line UT-SCC-2 (+TM) without or with specific MEK1/2 inhibitor PD98059 (PD; 10 μM), specific p38 inhibitor SB203580 (SB; 10 μM), or specific PI3k inhibitor LY294002 (LY; 10 μM), all of which were added 1 h before +TM. B, tumor fibroblasts were incubated for 24 h with 30% of +TM as described in A or with Ceramide (Cer; 50 μM) without or with MEK1/2 inhibitor PD98059 (PD) added at the concentrations indicated 1 h before +TM. A and B, total RNA was analyzed for expression of MMP-1 and GAPDH mRNAs by Northern blot hybridizations. The levels of MMP-1 mRNA were quantitated by densitometry and corrected for GAPDH mRNA levels and are shown below the panels relative to the levels in control cells (1).

![Fig. 7. TM-elicited induction of fibroblast MMP-1 expression is mediated by JNK2. A, embryonal fibroblasts from JNK2+/+ and JNK2−/− mouse were treated for 24 h with 50% of SCC tumor cell media, as indicated. B, JNK2+/+ and JNK2−/− fibroblasts were treated for 24 h with okadaic acid (OA; 10 ng/ml) or TGF-β (TGF; 5 ng/ml). A and B, aliquots (15 μg) of total RNA were analyzed for expression of murine collagenase-3 (mMMP-13) mRNA by Northern blot hybridizations. 28S rRNA was visualized by ethidium bromide staining as a loading control. Representative blots of two experiments, with fibroblasts derived from two distinct mouse strains, are shown. C, JNK2+/+ and JNK2−/− mouse embryonal fibroblasts were treated with 50% of conditioned medium of UT-SCC-2 cells (+TM) for the times indicated. Thereafter, cells were lysed to sample buffer, and activation of ERK1/2 (p-ERK) was determined by Western blot analysis using phosphospecific antibody. The filter was stripped and subjected to analysis of total JNK1 and JNK2 using specific antibodies. Cell lysates of HaCaT keratinocytes treated with EGF (20 ng/ml) for 20 min were used as a positive control for ERK1/2 activation. Interestingly, a recent study showed that inhibition of JNK2 expression by antisense oligonucleotides blocked EGF-induced transformation of human lung carcinoma cells (49), providing further evidence that specific inhibition of signaling via JNK2 may serve as a novel approach to inhibit tumor growth and invasion.]

A box contains certain conserved regulatory elements, including AP-1 and ETS-binding elements, similar to those in the human MMP-1 promoter, but in general, the two promoters do not show remarkable homology (23). However, the expression pattern of murine MMP-13 and human MMP-1 is similar in cutaneous wound repair in vivo because both are expressed by migrating keratinocytes and dermal fibroblasts (48), providing evidence for similar regulatory mechanisms for these MMPs, at least in fibroblasts and keratinocytes. Our results show that JNK2 is required for +TM-elicited induction of mouse MMP-13 expression, providing for the first time direct genetic evidence for the role of JNK signaling in the regulation of fibroblast MMP expression. In contrast, lack of JNK2 did not markedly alter the induction of mouse MMP-13 expression by okadaic acid or down-regulation of MMP-13 expression by TGF-β. This suggests that specific inhibition of JNK2 activity could be used to inhibit induction of MMP-1 expression by tumor cell-derived factors without interfering with regulation of physiological collagen turnover regulated by inflammatory cytokines and growth factors such as TGF-β.
INDUCTION OF STROMAL FIBROBLAST MMP-1 EXPRESSION

In conclusion, the results above show that low-passage tumor cells from SCCs of the head and neck secrete soluble factor(s), which dose-dependently activate MMP-1 expression in stromal fibroblasts. Furthermore, this effect is not due to the presence of TNF-α or IL-1β and is not dependent on MMP activity. Our results show that activation of fibroblast collagenolytic phenotype by SCC tumor cells is mediated by p38 MAPK and JNK2, which may serve as novel targets for therapy aimed at inhibiting malignant tumor invasion.

ACKNOWLEDGMENTS

The technical assistance of Hana Haavisto, Tarja Heikkilä, and Marita Potila is gratefully acknowledged. We also thank Dr. S. E. Bauer, P. Angel, J. Minna, and P. Fort for plasmids.

REFERENCES


Activation of Fibroblast Collagenase-1 Expression by Tumor Cells of Squamous Cell Carcinomas Is Mediated by p38 Mitogen-activated Protein Kinase and c-Jun NH$_2$-terminal Kinase-2

Jukka Westermarck, Songping Li, Panu Jaakkola, et al.

_Cancer Res_ 2000;60:7156-7162.

Updated version  Access the most recent version of this article at:  [http://cancerres.aacrjournals.org/content/60/24/7156](http://cancerres.aacrjournals.org/content/60/24/7156)

Cited articles  This article cites 46 articles, 18 of which you can access for free at:  [http://cancerres.aacrjournals.org/content/60/24/7156.full#ref-list-1](http://cancerres.aacrjournals.org/content/60/24/7156.full#ref-list-1)

Citing articles  This article has been cited by 4 HighWire-hosted articles. Access the articles at:  [http://cancerres.aacrjournals.org/content/60/24/7156.full#related-urls](http://cancerres.aacrjournals.org/content/60/24/7156.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.