Activin A enhances MMP-7 activity via the transcription factor AP-1 in an esophageal squamous cell carcinoma cell line

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Abstract. Activin A, a member of the transforming growth factor ß (TGF-ß) superfamily, is often overexpressed in solid carcinomas. We have previously reported that the expression of activin A is associated with lymph node metastasis in esophageal cancer. In the current study, our goal was to clarify the molecular mechanisms underlying the aggressive behavior of tumors expressing high levels of activin A. Using cDNA microarrays, the gene expression profile of a human esophageal carcinoma cell line (KYSE170) stably transfected with activin ßA (Act-ßA, a subunit of activin A) was compared with those of control human esophageal carcinoma cell lines. We found that expression of MMP-7 was higher in the Act-ßA transfectants than in the control cells. To reveal the mechanism of expression of MMP-7 mediated by activin A, we evaluated mRNA expression of MMP-7 and Act-ßA with or without activin A neutralizing antibody, using real-time PCR and Northern blot analysis. We also performed promoter analysis of the MMP-7 promoter and assessed c-Jun and Smad2/3 expression. MMP-7 expression in the transfectants was correlated with the level of Act-ßA expression and was reduced by activin A neutralizing antibody. The Act-ßA transfectants had higher MMP-7 promoter activity than control cells. MMP-7 promoter activity was not affected by mutation in the Smad binding site, while mutation of the AP-1 binding site did reduce activity. Moreover, the expression of c-Jun was increased in Act-ßA transfectants. These results indicate that activin A may modulate the expression of MMP-7 via AP-1 and not through Smad2/3.

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Abbreviations: TGF-ß, transforming growth factor beta; RT-PCR, reverse transcription polymerase chain reaction; MMP-7, matrix metalloproteinase 7; Act-ßA, activin beta A

Key words: activin A, MMP-7, AP-1, esophageal carcinoma

Introduction

We previously reported that overexpression of activin A in esophageal carcinoma tissues is associated with lymph node metastasis (1). Other studies have shown elevated activin A expression in pancreatic (2), prostate (3), ovarian (4) and colon (5) carcinomas. Moreover, patients with endometrial and cervical carcinomas have high serum levels of activin A (6), and activin A also stimulates the growth of BALB/c 3T3 fibroblasts, granulosa cells and ovarian carcinoma cell lines (7-9). Interestingly, mice deficient in inhibin A, which is a specific inhibitor of activin A, exhibit high levels of circulating activin and develop gonadal stromal tumors, raising the possibility that increased activin A expression may be tumorigenic under certain circumstances (10).

In the current study, we used an esophageal carcinoma cell line (KYSE170) stably transfected with activin ßA (Act-ßA), a subunit of activin A, and investigated the gene expression profiles using cDNA microarray analysis. We show that Act-ßA transfection significantly increased MMP-7 expression levels. MMP-7 is primordial member of the MMP family (11). It can degrade laminin (12), type IV collagen (13) and entactin (14), which are the main components of the basement membrane, and activate other important MMPs (MMP-1, MMP-2 and MMP-9) (15,16). It also inactivates α1-antitrypsin (17), which augments serine protease activity, and thus activates MMPs indirectly. We have demonstrated previously a correlation between MMP-7 and both tumor progression and metastasis in the human colon (18), gastric carcinoma (19) and esophageal carcinoma (20). The promoter of MMP-7 has a TATA box and binding sites for Ets, TCF, Smad and AP-1 (21). To better understand the role of activin A, we evaluated the expression of MMP-7 in the presence or absence of activin A neutralizing antibody. Moreover, we performed MMP-7 promoter assays with or without a mutation in Smad or AP-1 binding sites. Finally, we assessed the location of Smad2/3 and c-Jun proteins by immunoblot and immunofluorescent staining.

Materials and methods

Cell lines and activin-βA (Act-βA) transfection. The esophageal carcinoma cell line KYSE170 was obtained from the Department of Surgery and Surgical Basic Science, Kyoto University (Kyoto, Japan). KYSE170 was stably transfected

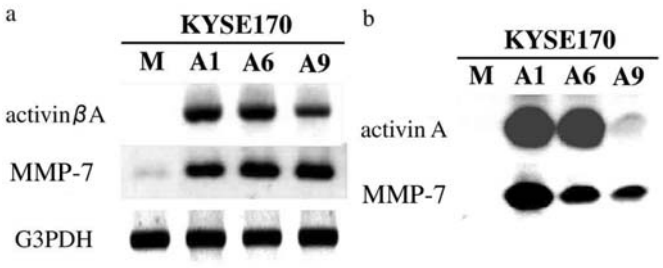

Figure 1. Expression of Act-ßA and MMP-7 assessed by (a) Northern blot and (b) immunoblot. (a) Expression level of 28S were quite similar in the three transfected clones (KYSE170A1, 6 and 9) and in the control cells (KYSE170M). Expression of Act-ßA was not detected in KYSE170M cells, but was observed in KYSE170A1, A6 and A9 cells. A faint band of MMP-7 was detected in KYSE170M cells. The stronger bands were detected in the three transfected clones (KYSE170A1, 6 and 9). (b) The immunoblot failed to detect expression of activin A and MMP-7 in KYSE170M cells. However, expression of activin A and MMP-7 is detected in KYSE170A1, 6 and 9 cells.

with Act-ßA. The PCR product of full-length Act-ßA cDNA was ligated into the pcDNA 3.1 expression vector (Invitrogen, Inc., Carlsbad, CA). It was confirmed that no mutation had occurred. Following transient transfection with Act-ßA using lipofectamine (Life Technologies, Inc., Rockville, MD), stable Act-ßA transfectants were selected using geneticin (G418; Life Technologies). The expression of Act-ßA mRNA was confirmed by both Northern blot and immunoblot (Fig. 1). All cell lines were maintained in RPMI-1640 (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal bovine serum (Equitech-Bio, Inc., Kerrville, TX). We isolated three single clones of Act-ßA cDNA transfectant which express different levels of activin A.

RNA extraction and Northern blotting

Total RNA isolation. Subconfluent cultured cell lines in a state of subconfluency were homogenized in 0.85 M guanidine thiocyanate, and total RNA was obtained by ultracentrifugation through a cesium chloride cushion, as described previously (22).

RNA analysis. Equal amounts (15 μg) of total cellular RNA were applied to lanes of a 1.2% agar-formaldehyde gel and electrophoresed for 7 h. The RNA samples were then transferred to nylon membranes (Hybond-N+; Amersham Pharmacia Biotech UK, Inc., Little Chalfont, UK). The membranes were cross-linked with 120,000 mJ/cm^2 using a UV Stratalinker 1800 (Stratagene, Inc., La Jolla, CA). After overnight hybridization at 42˚C, the blots were washed at a final stringency of 0.1X SSPE and 0.1% SDS at 65˚C. Autoradiography was performed at room temperature with an identifying screen using a Bio-Image analyzer BAS 2500 (Fuji Photo Film Co., Tokyo, Japan).

Probe preparation. The DNA probes for the open reading frame of Act-ßA were subcloned into the pCR II cloning vector (Invitrogen), digested with *Eco*RI, loaded onto 1.0% agar gels and electrophoresed. The inserts were extracted using a gel extraction kit (Takara, Inc., Otsu, Japan) and labeled with [^{32}P]-deoxycytosine triphosphate using a random-primed DNA labeling kit (Takara). A DNA probe for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was made as previously described (23).

Quantitative reverse transcription polymerase chain reaction. cDNAs were synthesized from 8 μg of total RNA in 30 μl reaction mixtures, as described previously (24). The products were loaded onto 1.2% agarose gels with 400 ng/ml ethidium bromide and electrophoresed. Real-time, no-nested PCR for Act-ßA and MMP-7 cDNA (bp) was performed using a LightCycler thermal cycler system (Roche Diagnostics, Basel, Switzerland), according to the manufacturer's instructions. The following primers were used: Act-ßA sense, 5'-TGG GCA AGA AGA AGA AGA AAG-3', and antisense, 5'-CGC AGT AGT TGG CAT GAT AGC-3'. For MMP-7, the primers were: sense, 5'-TAC AGT GGG AAC AGG CTC AGG-3', and antisense, 5'-GGC ACT CCA CAT CTG GGC T-3'. As previously described (25), for G3PDH we used sense, 5'-TTG GTA TCG TGG AAG GAC TCA-3', and antisense, 5'-TGT CAT CAT ATT GGG CAG GTT T-3'. For PCR, 1 μl of RNA was placed into a 19 μl reaction volume containing 0.67 μl primer, 1.2 μl 25 mM $MgCl_2$, and 2 μl LightCycler-FastStart DNA Master SYBR-Green I mix (Roche Diagnostics). The protocol included a polymerase activation step at 95˚C for 600 sec followed by 40 cycles that each included 95˚C denaturation for 20 sec, 55˚C annealing for 20 sec and 72˚C extension for 20 sec. Negative controls were run concomitantly to confirm that the samples were not cross-contaminated. A sample with 1 μl diethylpyrocarbonate-treated water instead of RNA was concomitantly examined for each of the reactions described above. To confirm the amplification specificity, the PCR products were subjected to a melting curve analysis. A positive control using RNA obtained from the HT29 cell line was also performed for each LightCycler run.

Immunoblot. Total protein was extracted from the esophageal carcinoma cell lines using 1X sample buffer, as described elsewhere (25). Identical aliquots (30 μg) of total protein were applied to several 10% acrylamide gradient gels. After electrophoresis, the samples were electroblotted onto a polyvinylidene difluoride membrane (Immobiline; Millipore, Inc., Bedford, MA) at 0.5 A for 0.5 h at 4˚C. Act-ßA and MMP-7 were detected using mouse monoclonal primary antibodies to Act-ßA (R&D systems, Minneapolis, MN) and MMP7 (Transduction Laboratories, Lexington, KY) at a dilution of 1:1000. The blots were developed using horseradish peroxidase-linked anti-mouse immunoglobulin whole antibody (Promega, Inc., Madison, WI). Signals for Act-ßA and MMP-7 were detected using Supersignal (Pierce, Inc., Rockford, IL). Pre-stained high molecular weight markers were also run on the gels (Amersham Pharmacia Biotech UK).

cDNA microarray. A cDNA microarray 'Human cancer chip version 2.1' (Takara Biochemicals, Tokyo, Japan) was used and the analysis was performed as follows. For competitive hybridization, cDNAs carrying a Cy5- or Cy3-dUTP (Amersham Pharmacia, Piscataway, NJ) label were

constructed from 15 μg of total cellular RNA of control (mock-transfected) cells (KYSE170M) and Act-ßA transfectants (KYSE170A1) using an RNA labeling kit (Takara Biochemicals). The microarray was then hybridized to the probes. After overnight hybridization at 65˚C, the slide was twice washed in 2X SSC and 0.5% SDS for 30 min at 55˚C, then washed in 2X SSC and 0.5% SDS for 5 min at 65˚C, and then in 0.05X SSC and 0.5% SDS for 5 min at room temperature. The slides were then scanned using a GMS418 (Molecular Dynamics, Sunnyvale, CA) with separate measurements of the sample intensities for Cy3 and Cy5. The intensity of each hybridization signal was evaluated photometrically using Imagene software (BioDiscovery, Marina Del Rey, CA), and normalized to the average signal of a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (G3PDH). The Cy3:Cy5 ratio for each sample was calculated by averaging the spots, and the cut-off value for each expression level was calculated according to the background noise. For this cut-off, we used an expression level of >100 where the fluctuation was below a critical value (1.0), because other genes (those with low expression) are embedded in the background noise. Up-regulated and down-regulated genes in the Act-ßA transfectants were defined as those having a Cy3:Cy5 signal ratio >2.0, or <0.50, respectively.

Promoter assay. MMP-7 promoters with or without a mutation in the Smad binding site or the AP-1 binding site (established by Crawford *et al*) were kindly provided by Dr Lynn Matrisian (26). A transfection mixture was created by incubating 1 mg of firefly luciferase reporter with 10 ng of the *Renilla* luciferase internal control, SV40-RL (Promega), and 1 mg each of the expression vectors indicated below. The volume was brought to 200 μl using OptiMEM (Invitrogen), and 15 μl of Superfect (Qiagen) transfection reagent was added and mixed by pipetting. After a 15-min incubation, 1 ml of DMEM containing 10% FBS was added, the contents were mixed, and 400 μl was distributed to each of 3 wells of a 24-well plate, each well containing KYSE170M or KYSEA1, 6, 9 plated 24 h prior to transfection. Total DNA in the transfection mixture was kept constant by including the same empty vectors as those that contained the cDNAs being expressed. The transfection mixture was removed from the cells 2-3 h after addition and replaced with DMEM with 10% FBS. Luciferase activity was determined using the Dual Luciferase kit (Promega) 16-24 h post transfection by lysing in 50 μl of passive lysis buffer and assaying both firefly and *Renilla* luciferase activities in the same 30 ml aliquot of lysate.

Fold-induction was determined by first normalizing each firefly luciferase value to the *Renilla* luciferase internal control, averaging the normalized values, and dividing by the mean value of the firefly reporter cotransfected with empty vectors only. Normalized relative light units (RLUs) were determined by normalizing each firefly luciferase value to the highest *Renilla* luciferase value in a given experiment by the following formula: (highest *Renilla* luciferase value in the experiment/*Renilla* luciferase value of the individual sample) x firefly luciferase value of the same sample. Whether using fold-induction or normalized RLUs, each experiment was repeated as noted in the figure legends and the means and standard errors were calculated using Microsoft Excel.

Quantitation and statistical methods. The significance of difference between the variables was tested by Student's t-test or Fisher's exact probability test. Correlation between the variables was tested using StatView J-4.5 software (SAS Inst., Cary, NC).

Results

Activin A expression in Act-ßA. The open reading frame of Act-ßA cDNA subcloned in pcDNA 3.1 was transfected into the KYSE170 esophageal carcinoma cell line. We isolated three single clones of Act-ßA transfected cells (KYSE170A1, 6, 9) and one single clone of control vector transfected cells (KYSE170M). Expression of Act-ßA mRNA was shown by Northern blot (Fig. 1a) and expression of activin A itself was shown by Western blot (Fig. 1b). These results confirmed that the transfection of Act-ßA was successful and that the full-length form of activin A was produced by the Act-ßA transfectants.

Identification of elevated MMP-7 expression in Act-ßA transfectants. A cDNA microarray analysis was performed on the transfected population-(KYSE170A1) and on control cells (KYSE170M). Tables I and II show the genes which were expressed at higher or lower levels in transfected cells (KYSE 170A1) relative to control cells (KYSE 170M). MMP-7 mRNA expression in KYSE 170A1 showed a 20.17-fold-increase relative to that observed in KYSE170M. The expression of MMP-7 mRNA and protein in three single clones of transfectants (KYSE170A1, A6, A9) was higher than in the control cells (KYSE170M) (Fig. 1a and b).

Correlated expression of Act-ßA and MMP-7: activin A neutralizing antibody. From the Northern blot and immunoblot, it was apparent that expression of MMP-7 mRNA and protein were higher in transfectants than in control cell lines (Fig. 1a and b). A quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed on mRNA derived from three cloned transfectants and control cells using real-time PCR (Fig. 2a and b). The expression levels of Act-ßA, MMP-7 and G3PDH were calculated relative to their expression in a positive control cell line, HT29. To adjust the expression levels of Act-ßA and MMP-7 in each sample, we used the expression levels of G3PDH in the sample and the control, using the following equations: the value for Act-ßA expression = log (Act-ßA/G3PDH) while the value for MMP-7 expression = log (MMP-7/G3PDH), as described previously (25). We then examined the correlation between the values for Act-ßA and MMP-7 (Fig. 2c). This result indicates that the expression levels of Act-ßA mRNA and MMP-7 mRNA were significantly correlated.

The expression of MMP-7 mRNA in KYSE170A1 was assessed in the presence and absence of activin A neutralizing antibody. The expression of MMP-7 mRNA decreased in the presence of activin A neutralizing antibody in a dose dependent manner, as revealed by Northern blot analysis

Table I. Higher expression genes in activin A transfectant cells.

Symbol	Gene name	Protein encorded	Accession no.	Fold
MMP7	Matrix metalloproteinase 7 (matrilysin, uterine)	AAV40839	AY795972	20.2
KRT13	Keratin 13	CAA32786	X14640	4.82
CCNG2	Cyclin G2	NP_004345	NM_004354	4.69
LTA	Lymphotoxin α (TNF superfamily, member 1)	AAH34729	BC034729	4.49
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	NP_003801	NM_003810	4.02
MMP13	Matrix metalloproteinase 13 (collagenase 3)	NP_002418	NM_002427	3.92
KRT6B	Keratin 6B	AAH34535	BC034535	3.44
TGFBI	Transforming growth factor, ß-induced, 68 kD	AAH04972	BC004972	3.06
CDH8	Cadherin 8	NP_001787	NM_001796	2.77
TNFRSF10	Tumor necrosis factor receptor superfamily, member 10b	NP_003833	NM_003842	2.74
COL17A1	Collagen, type XVI, α 1	NP_000485	NM_000494	2.65
PGK1	Phosphoglycerate kinase 1	AAI04838	BC104837	2.54
ALDOA	Aldolase A, fructose-bisphosphate	AAH04333	BC004333	2.51
IFITM2	Interferon induced transmembrane protein 2 (1-8D)	NP_006426	NM_006435	2.43
FDFT1	Farnesyl-diphosphate farnesyltransferase 1	AAH29641	BC029641	2.27
PTGES	Prostaglandin E synthase	AAH08280	BC008280	2.10
MIHC	Apoptosis inhibitor 1	AAC83232	AF070674	2.10
SOD2	Superoxide dismutase 2, mitochondrial	AAH12423	BC012423	2.08
TGFB1	Transforming growth factor, ß 1	AAH22242	BC022242	2.02
TUBB	Tubulin, ß polypeptide	NP_821133	NM_178014	2.02
TOP2A	Topoisomerase (DNA) II α (170 kD)	NP_001058	NM_001067	2.00

Table II. Lower expression genes in activin A transfectant cells.

Symbol	Gene name	Protein encorded	Accession no.	Fold
CASP8	Caspase 8, apoptosis-related cysteine protease	NP_001219	NM_001228	0.25
CD8B1	CD8 antigen, ß polypeptide 1 (p37)	AAX42824	AY890886	0.31
AREG	Amphiregulin (schwannoma-derived growth factor)	AAX42908	AY890973	0.32
RRAD	Ras-related associated with diabetes	AAH57815	BC057815	0.36
NF2	Neurofibromin 2 (bilateral acoustic neuroma)	AAH03112	BC003112	0.36
VDR	Vitamin D (1,25-dihydroxyvitamin D3) receptor	NP_000367	NM_000376	0.36
KISS1	KiSS-1 metastasis-suppressor	NP_002247	NM_002256	0.40
VCAN	Chondroitin sulfate proteoglycan 2 (versican)	NP_004376	NM_004385	0.40
PRDX3	Peroxiredoxin 3	AAH59169	BC059169	0.41
ITGA8	Integrin, α 8	NP_003629	NM_003638	0.41
CDC16	CDC16 (cell division cycle 16, S. cerevisiae, homolog)	CAM22287	AL160396	0.44
PIGF	Phosphatidylinositol glycan, class F	NP_775097	NM_173074	0.44
SMN1	Survival of motor neuron 1, telomeric	AAH62723	BC062723	0.48
MKK4	Mitogen-activated protein kinase kinase 1	AAC24130	AH006187	0.49
CDC2L5	Cell division cycle 2-like 5 (cholinesterase-related cell division controller)	CAC10401	AJ297710	0.49
RBL2	Retinoblastoma-like 2 (p130)	NP_005602	NM_005611	0.49

(Fig. 3a) and real-time PCR (Fig. 3b). These results indicate that the expression of MMP-7 mRNA was attenuated by neutralizing of activin A activity.

The decrease promoter activity of MMP-7 promoter with mutation of ap-1 binding site. We assessed the promoter activity of the human MMP-7 promoter (HMAT) in

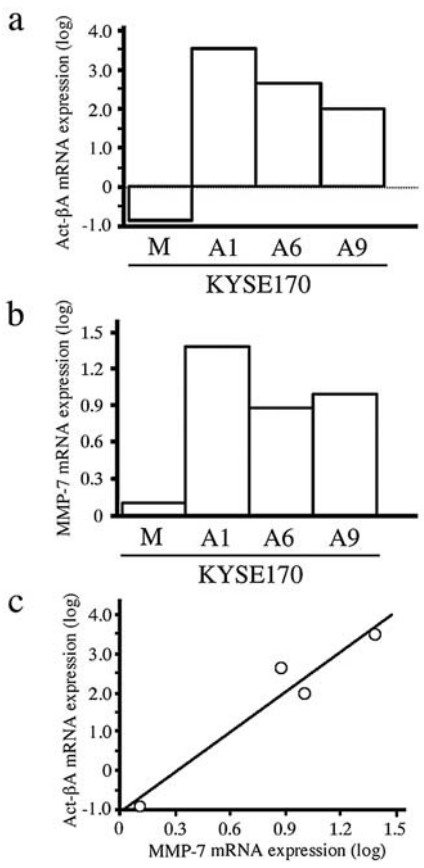

Figure 2. Expression of Act-ßA mRNA and MMP-7 mRNA. Expression was assessed by the quantitative reverse transcription polymerase chain reaction method. (a) The relative expression of Act-ßA mRNA in KYSE-170M1, A1, 6 and 9 cells compared to control cells (KYSE 70). (b) The relative expression of MMP-7 mRNA in KYSE170M1, A1, 6 and 9 cells compared to control cells (KYSE70). (c) The correlation between Act-ßA and MMP-7 expression. The correlation coefficient is 0.693.

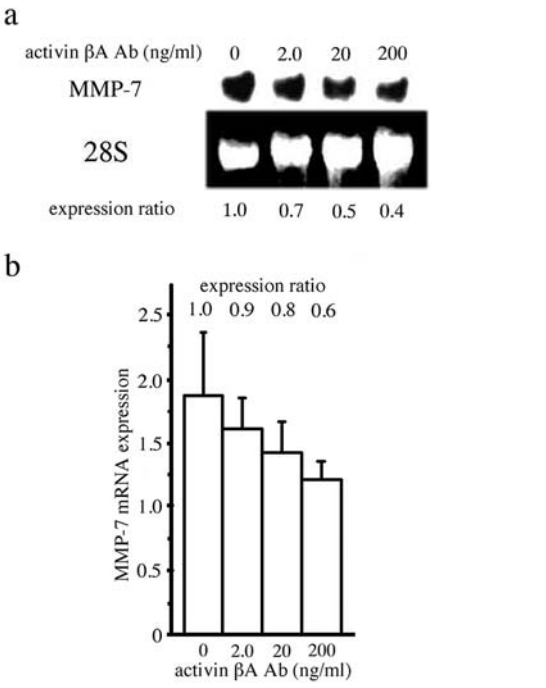

Figure 3. Effect of anti-human activin A neutralizing antibody on KYSE170A1. Expression of MMP-7 mRNA was inhibited in a dose-dependent fashion by activin A neutralizing antibody. (a) Northern blotting, upper number indicates the dose of anti-human activin A neutralizing antibody and lower number indicates the mRNA expression ratio compared to KYSE170M. (b) Quantitative RT-PCR, upper number indicates the expression ratio compared to KYSE170M.

KYSE170A1, A6, A9 and KYSE170M. Promoter activity was elevated in Act-ßA transfectants compared with control cells (Fig. 4a). To identify the transcription factor that was modulated by activin A, we used HMAT promoters with a mutation in either the Smad binding site or the AP-1 binding site. The promoter activities of HMATs with wild-type or mutant of Smad binding sites were similar in Act-ßA transfectants and control cells (KYSE170A1). However, the promoter activity of HMAT with a mutant of AP-1 binding site was lower than that of wild-type HMAT (Fig. 4b).

Nuclear accumulation of c-Jun in Act-ßA transfectants. To evaluate the expression of c-Jun and Smad2 in Act-ßA transfectants (KYSE170A1) and control cells (KYSE170M), we performed immunofluorescent staining and immunoblot analysis. The immunofluorescent stain revealed nuclear accumulation of Smad2/3 and higher expression of c-Jun in nuclei inKYSE170A1 (Fig. 5a). In the immunoblot analysis, the amount of Smad2/3 in nuclei of Act-ßA transfectants (KYSE170A1, 6, 9) was elevated, but the total amount of Smad2/3 was similar to that of control cells (KYSE170M) (Fig. 5b). Furthermore, c-Jun in Act-ßA transfectants (KYSE170A1, 6, 9) was expressed at higher levels than in control cells (KYSE170M) (Fig. 5b). These results indicate

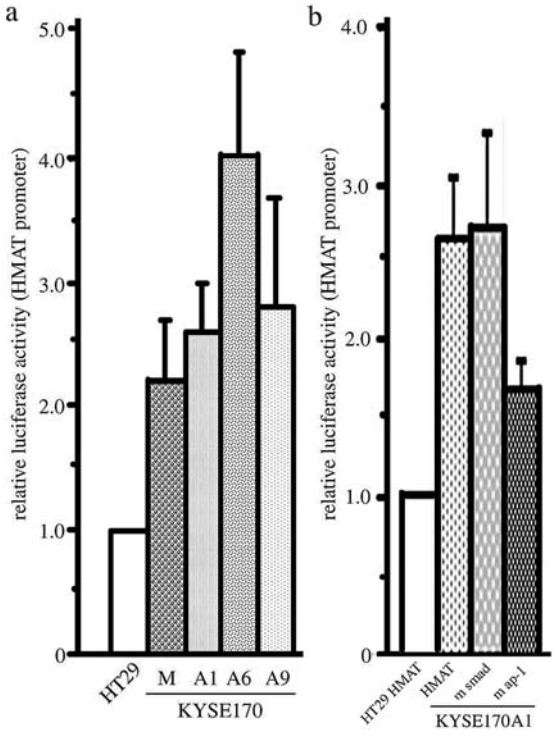

Figure 4. The reporter assay for human MMP-7. (a) The relative luciferase activities in KYSE170M, A1, 6, 9 and HT29 are assessed. Cloned Act-ßA transfectants (KYSE170A1, 6 and 9) possessed higher luciferase activities than KYSE170M. (b) Evaluation of the promoter activity of MMP-7 with or without mutation of the Smad binding site or the AP-1 binding site. The luciferase activity of the MMP-7 promoter with a mutant of AP-1 binding site is reduced compared to the promoter activity of MMP-7 with or without mutation in Smad binding site.

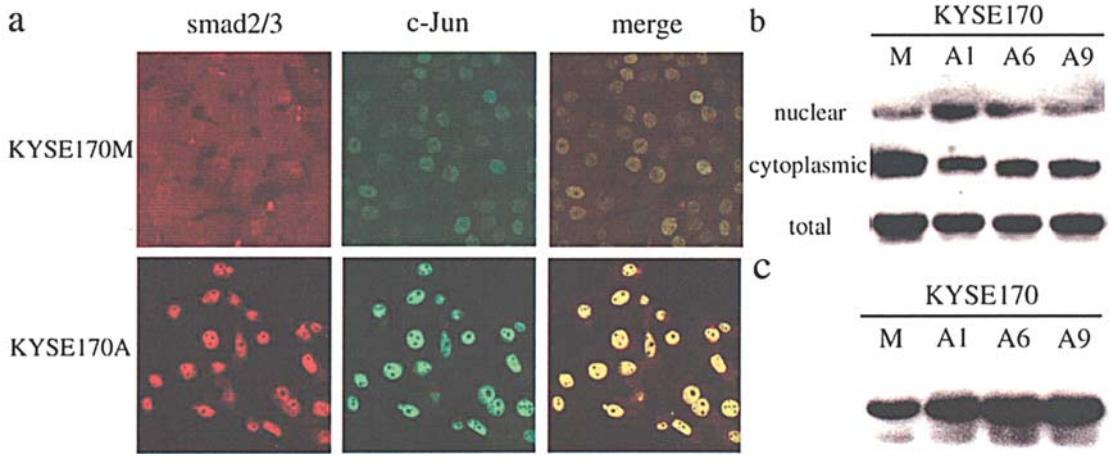

Figure 5. Effect of activin A on specific transcription factors. (a) Immunofluorescent staining of Smad2/3 and c-Jun in KYSE170M and A1. Strong nuclear stain of Smad2/3 and c-Jun was observed in KYSE170A1 compared to KYSE170M. (b) Immunoblot of Smad2/3 was performed using total, cytoplasmic or nuclear protein extracts of KYSE170M, A1, 6 and 9. In all Act-ßA transfectant clones (KYSE170A1, 6, 9), nuclear Smad2/3 is abundant compared to KYSE170M. (c) Immunoblot analysis of c-Jun. The expression of c-Jun is higher in KYSE170A1, 6 and 9 than in KYSE170M.

that activin A mediates the expression of c-Jun in nuclei and may enhance the promoter activities of wild-type HMAT.

Discussion

The elevated expression of activin A in solid carcinomas is related to cancer aggressiveness. To reveal this mechanism, we performed cDNA microarray analysis using mRNA samples from stable activin-ßA (Act-ßA) transfectant cells (KYSE170A1) and mock control cells (KYSE170M). The cDNA microarray analysis showed that MMP-7 expression was elevated in KYSE170A1. We showed that the MMP-7 expression level was directly correlated with the level of the Act-ßA expression in transfectants and control cells. Furthermore, MMP-7 expression in KYSE170A1 was attenuated by anti-human activin A neutralizing antibody. These results indicated that activin A augments MMP-7 expression.

We assumed that the promoter activity of HMAT was not strong. However, it was possible to evaluate the luciferase activities in HT29, KYSE170M, A1, A6 and A9. The luciferase activities of all transfectant clones were greater than that of KYSE170M. However, values were somewhat low and statistical significance could not be demonstrated. The reporter assay showed that there was no remarkable difference between the promoter activity of MMP-7 with or without a mutation of the Smad binding site. However, the difference was observed in MMP-7 promoter activity when the AP-1 binding site was altered. Several reports have shown that activin A and/or TGF-ß modulate c-Jun expression (27,28) and that of c-Jun/AP-1 regulates MMP-7 expression (29-31). In the immunofluorescence assay, the c-Jun signal was higher in the transfectants compared to control cells, but a faint c-Jun signal was observed in the control cells. These results indicate that while activin A may not be absolutely required for MMP-7 expression, it does appear to enhance MMP-7 expression through the AP-1 binding site. MMP-7 expression in solid carcinomas is associated with the cancer aggressiveness. Our data may indicate that MMP-7 expression mediated by activin A plays one of the important roles of esophageal carcinoma aggressiveness.

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