

Expression of the *Elm1* Gene, a Novel Gene of the CCN (Connective Tissue Growth Factor, Cyr61/Cef10, and Neuroblastoma Overexpressed Gene) Family, Suppresses In Vivo Tumor Growth and Metastasis of K-1735 Murine Melanoma Cells

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Summary

We previously isolated a partial cDNA fragment of a novel gene, *Elm1* (expressed in low-metastatic cells), that is expressed in low-metastatic but not in high-metastatic K-1735 mouse melanoma cells. Here we determined the full-length cDNA structure of *Elm1* and investigated the effect of *Elm1* expression on growth and metastatic potential of K-1735 cells. The *Elm1* gene encodes a predicted protein of 367 amino acids showing ~40% amino acid identity with the CCN (connective tissue growth factor [CTGF], Cyr61/Cef10, neuroblastoma overexpressed gene [Nov]) family proteins, which consist of secreted cysteine-rich proteins with growth regulatory functions. *Elm1* is also a cysteine-rich protein and contains a signal peptide and four domains conserved in the CCN family proteins. *Elm1* was highly conserved, expressed ubiquitously in diverse organs, and mapped to mouse chromosome 15. High-metastatic K-1735 M-2 cells, which did not express *Elm1*, were transfected with an *Elm1* expression vector, and several stable clones with *Elm1* expression were established. The in vivo growth rates of cells expressing a high level of *Elm1* were remarkably slower than those of cells expressing a low level of *Elm1*. Metastatic potential of transfectants was reduced in proportion to the level of *Elm1* expression. Thus, *Elm1* is a novel gene of CCN family that can suppress the in vivo growth and metastatic potential of K-1735 mouse melanoma cells.

It is now widely accepted that malignant tumors contain heterogeneous populations of cells with regard to metastatic potential (1). The process of cancer metastasis consists of linked sequential steps, including invasion, detachment, intravasation, circulation, adhesion, extravasation, and growth in distant organs (2). Thus, high- and low-metastatic cells in a tumor should be different each other with respect to several biological properties, such as invasiveness, adhesiveness, motility, and proliferation potential. There is much evidence to support the concept that each discrete step of metastasis is regulated by transient or permanent changes at the DNA, messenger RNA (mRNA),¹ and/or protein levels in differ-

ent genes (3–5). By using the mRNA differential display method, we previously identified a partial 3' cDNA fragment of a novel gene, *Elm1* (for expressed in low-metastatic type 1 cells), that is expressed in low- but not in high-metastatic K-1735 murine melanoma cells (6). *Elm1* was also differentially expressed between high- and low-metastatic B16 murine melanoma cells. A partial *Elm1* cDNA fragment of 211 nucleotides showed no significant homology to any recorded sequence in the DDBJ/GenBank/EMBL DNA databases.

Here we determined the full-length cDNA structure of

cells; Fisp, fibroblast-inducible secreted protein; IBP, insulin-like growth factor binding protein; mRNA, messenger RNA; MSM, *Mus musculus molossinus*; Nov, neuroblastoma overexpressed gene; NovM, mouse Nov; nt, nucleotide; TSP1, thrombospondin type 1 repeat; VWC, von Willebrand factor type C repeat.

¹Abbreviations used in this paper: bFGF, basic fibroblast growth factor; C-23, clone 23; CCN, CTGF, Cyr61/Cef10, and Nov; CT, COOH-terminal; CTGF, connective tissue growth factor; Elm, expressed in low-metastatic

the *Elm1* gene. The *Elm1* gene encoded a novel type of CCN (connective tissue growth factor [CTGF], Cyr61/Cef10, and neuroblastoma overexpressed gene [Nov]) family proteins that consisted of secreted cysteine-rich molecules, CTGF, Cyr61/Cef10, and Nov (7–11). CTGF and Cyr61 were induced within minutes of stimulation by serum or growth factors (10, 12) and have a growth regulatory function in fibroblasts or endothelial cells (13, 14). Nov is a protooncogene isolated from myeloblastomatosis-associated virus-induced nephroblastoma (9). Because of the high level of sequence similarities between *Elm1* and the other members of the CCN family, it is likely that *Elm1* has a function to regulate the growth of normal and/or cancerous cells. Since *Elm1* gene expression was high in low-metastatic tumor cells and declined with increasing metastatic potential in two rodent experimental systems, we investigated the biological effects of the *Elm1* gene on in vitro growth, in vivo growth, and metastatic potential of K-1735 murine melanoma cells. By introduction of an *Elm1* expression vector into high-metastatic K-1735 M-2 cells that did not express endogenous *Elm1*, it was revealed that expression of *Elm1* can inhibit tumor growth in vivo as well as metastasis of K-1735 melanoma cells.

Materials and Methods

Cell Lines. K-1735-derived mouse melanoma cell lines were obtained from I.J. Fidler (University of Texas, Houston, TX; reference 15). Clone 23 (designated C-23) was classified as low metastatic, whereas clone M-2 was high metastatic in syngeneic recipients (16, 17). Serum stimulation was performed using BALB/c 3T3 cells.

Cell Culture and mRNA Isolation. K-1735 cells and BALB/c 3T3 cells were maintained in tissue culture in the DME-10 (DME supplemented with 10% fetal calf serum, sodium bicarbonate solution, L-glutamine, and penicillin-streptomycin). Cells were maintained on plastic and were incubated in 5% CO₂/95% air at 37°C. Cells at 70% confluency were harvested and subjected to mRNA extraction. mRNA was isolated using Fast Track mRNA Isolation Kit (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's recommendations.

Screening of cDNA Library. To isolate full-length cDNA clones, cDNA libraries from K-1735 C-23 (low-metastatic clone) constructed in lambda ZAP II (Stratagene Corp., La Jolla, CA) using cDNA Synthesis System Plus (Amersham Corp., Arlington Heights, IL) was screened with a cloned cDNA fragment of *Elm1* that was previously isolated by an mRNA differential display (6). Isolated clones were sequenced by the A.L.F. DNA Sequencer II with the AutoRead Sequencing Kit (Pharmacia Biotech, Piscataway, NJ). DNA sequences were aligned, examined for open reading frames, and compared with DNA sequences in the DDBJ/GenBank/EMBL and amino acid sequence in the SWISS-PROT and PIR protein databases using the FASTA and BLAST programs.

Northern and Southern Blot Analyses. 3 µg of poly(A)⁺ RNA were size fractionated on a denaturing formaldehyde agarose gel (1.0%) and transferred onto Hibond-N⁺ membrane (Amersham Corp.). Mouse multiple tissue Northern (MTN) blot, containing 2 µg of poly(A)⁺ RNA from a variety of tissues, and ZOO BLOT, containing 8 µg of EcoRI-digested genomic DNA from various species, were obtained from Clontech (Palo Alto, CA). Northern

blot hybridization was performed at 42°C for 24 h with hybridization buffer containing 50% formamide, 5× NET (750 mM NaCl, 5 mM EDTA, and 75 mM Tris-HCl), 0.5% dry milk, 0.4% SDS, 10% dextran sulfate, and 0.2 mg/ml salmon sperm DNA. Then the membrane was washed with 0.1× SSC and 0.1% SDS at 65°C for 60 min. Southern blot hybridization was performed at 37°C with hybridization buffer described above, and the membrane was washed with 0.1× SSC and 0.1% SDS at 55°C. To confirm the amounts of mRNA loaded in each lane, the blots were hybridized afterwards with a human β-actin probe (Clontech). A DNA probe for the *Elm1* gene corresponded to nucleotides 33–2399 of the *Elm1* cDNA fragment. A DNA probe corresponding to nucleotides 337–1119 of the *Cyr61* mRNA (DDBJ/GenBank/EMBL accession number: M32490) was synthesized by reverse transcription PCR.

Chromosome Mapping of the *Elm1* Gene. An interspecific backcross panel formed from (DBA/2J × MSM)F1 × DBA/2J (MSM, *Mus musculus molossinus*) was used for mapping of the *Elm1* gene (18). Genotypes of *Elm1* for 138 individuals in this panel were determined by RFLP observed in PCR-amplified products. A genomic DNA fragment of 1,193 bp in the *Elm1* locus was amplified by primers 5'-CGATATCTTTGCTGACTTGG-3' (sense strand) and 5'-CTGAGGCTGTAAAGTAGGTC-3' (antisense strand), corresponding to nucleotides 1223–1242 and 2396–2415, respectively. The restriction enzyme *Sau3AI* yielded an easily distinguishable polymorphism between two parental strains, MSM and DBA/2J. The data generated in this study was analyzed (Map Manager v 2.5.6; Roswell Park Cancer Institute, NY; reference 19).

Serum Stimulation of BALB/c 3T3 Cells. Quiescent BALB/c 3T3 cells were prepared by growth in DME-10 to confluence followed by incubation in DME-0.5 (0.5% serum) for 2 d. For stimulation of quiescent cells, the medium was changed to DME-20 (20% serum).

Construction of Expression Vector and DNA Transfection. The pcDNA3 expression vector was purchased from Invitrogen. A cDNA fragment of *Elm1* (146–1,309 nucleotides [nt]) consisting of 28 nts of the 5'-untranslated region, 1,101 nts of the coding region, and 35 nts of the 3'-untranslated region was amplified using the primer set *Elm1*-B (146–165 nts; GTAGCTCCTGTGACGCTGAC) and *Elm1*-C (complemented 1,290–1,309 nts; GCATGGAACCTTTACCCTGAG), and ligated to the pcDNA3 vector at the BamHI site (pcDNA3-*Elm1*) in the direction of plus strand. K-1735 M-2 cells were transfected with pcDNA3-*Elm1* using LipofectAMINE reagent (GIBCO BRL, Gaithersburg, MD). After 16 h, the medium was changed to DME-10. At 38 h of transfection, G418 selection was imposed (800 µg/ml; Geneticin; GIBCO BRL). G418-resistant cells were cloned by using the penicillin cap method and maintained in the medium containing G418. Cell clones resistant to G418 were assayed for the expression of *Elm1* mRNA by Northern blotting.

Cell Growth and Tumorigenicity. K-1735 M-2, C-23, and transfectants were seeded at the density of 5 × 10³ cells/ml in a 24-well plate. The cells were counted every day from days 1 to 6. 5-wk-old female C3H/HeN mice were obtained from the Animal Production of Japan Kurea Corporation (Tokyo, Japan). Tumorigenicity was examined by injecting 10⁶ cells/0.2 ml into the subcutis of the mice (*n* = 5). The length and width of each tumor were recorded three times per week. The tumor volume (V) was calculated by the formula V = 1/2 × length × (width)².

Experimental Metastasis Assay. Metastatic potential of the cells was measured by the quantitative lung colony assay as described by Fidler et al. (20). In brief, the cells were injected into the tail vein of 5-wk-old female C3H/HeN mice at the density of 10⁵

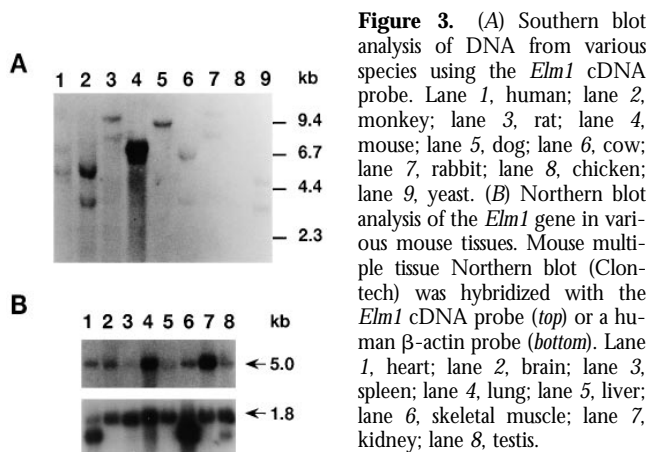


Figure 3. (A) Southern blot analysis of DNA from various species using the *Elm1* cDNA probe. Lane 1, human; lane 2, monkey; lane 3, rat; lane 4, mouse; lane 5, dog; lane 6, cow; lane 7, rabbit; lane 8, chicken; lane 9, yeast. (B) Northern blot analysis of the *Elm1* gene in various mouse tissues. Mouse multiple tissue Northern blot (Clontech) was hybridized with the *Elm1* cDNA probe (top) or a human β -actin probe (bottom). Lane 1, heart; lane 2, brain; lane 3, spleen; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, testis.

Cyr61, Fisp12, NovM in each of the four domains (Fig. 2). Similarities of the amino acid sequence outside the four domains were insignificant for any of the three subclasses. A dendrogram indicates that *Elm1* is not an orthologue of other CCN family members (Fig. 2 B).

The *Elm1* gene is conserved among different species (Fig. 3 A), and highly expressed in the kidney and lung, and at lower levels in the heart, brain, spleen, liver, skeletal muscle, and testis (Fig. 3 B). The *Elm1* locus was mapped to chromosome 15, between the *D15Mit17* and *D15Mit3* loci, which is not a site of known mouse CCN family genes (22, 23; Fig. 4).

Quiescent BALB/c 3T3 cells were stimulated by serum, and the expression of *Elm1* and *Cyr61* were examined by Northern blot analysis (Fig. 5). *Elm1* was not induced within 30 min, but was induced after 3 h of serum stimulation. Although the amount of β -actin mRNA in lane 1 quantified by densitometric analysis was about half of that in lane 3, the relative level of *Elm1* expression in lane 3, that is, the ratio of *Elm1* mRNA over β -actin mRNA, was seven times higher than that in lane 1. On the other hand, *Cyr61* expression was markedly induced within 30 min (Fig. 5).

Effects of *Elm1* Expression on the Growth and Metastasis of K-1735 Cells. A full-length *Elm1* cDNA was transfected into K-1735 M-2 cells using the pcDNA3 vector, and several G-418 resistant clones were tested for continued expression of *Elm1* mRNA by Northern blot analysis (Fig. 6 and Table 1). M-2.*Elm1*.23-1 cells showed the highest level of *Elm1* mRNA expression among clones examined. M-2.*Elm1*.20-3 and M-2.*Elm1*.6-2 cells showed a similar level of *Elm1* expression to endogenous *Elm1* expression in low-metastatic K-1735 C-23 cells. M-2.*Elm1*.0-1 and M-2.*Elm1*.20-2 cells showed a lower level of *Elm1* expression than endogenous *Elm1* expression in K-1735 C-23 cells. M-2.*Elm1*.8-3 cells did not express detectable levels of *Elm1*, but expressed Neo mRNA. Thus, the level of *Elm1* expression in the cloned cells was in the order of M-2.*Elm1*.23-1 > M-2.*Elm1*.20-3 > M-2.*Elm1*.6-2 > K-1735 C-23 > M-2.*Elm1*.0-1 > M-2.*Elm1*.20-2 > M-2.*Elm1*.8-3 > K-1735 M-2 (Table 1).

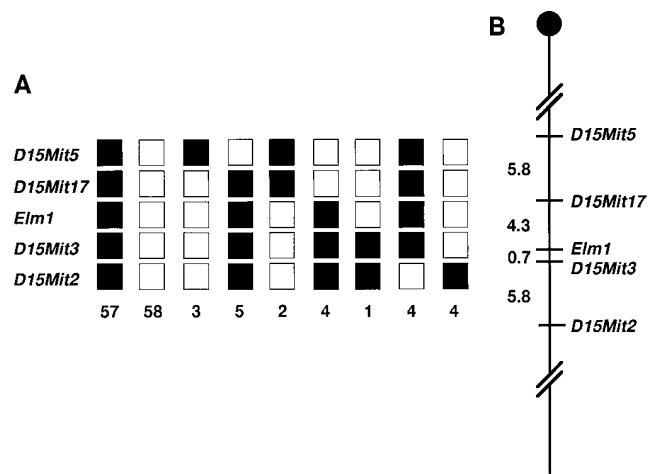


Figure 4. Linkage analysis of the *Elm1* locus on mouse chromosome 15. (A) Haplotype data of 138 progenies of (DBA/2J \times MSM)F1 \times DBA/2J for the loci flanking the *Elm1* locus. The microsatellite and *Elm1* loci are listed at the left. Each column represents a chromosomal haplotype identified in the progenies. Filled box, MSM allele; open box, DBA/2J allele. The number of progenies for each haplotype is listed at the bottom of each column. (B) A genetic map around the *Elm1* locus constructed from the haplotype data. Recombination frequencies expressed as genetic distance in centiMorgan are shown on the left.

We examined the in vitro growth properties of K-1735 M-2 and transfectants. Transfectants that express large amounts of *Elm1*, M-2.*Elm1*.20-3, and M-2.*Elm1*.23-1 cells showed slightly increased population doubling time and decreased saturation density compared to those of K-1735 M-2 cells (Table 1). Morphological diversities were not observed among the clones in association with the level of *Elm1* expression. We next injected the clones subcutaneously into female C3H/HeN mice to evaluate the effect of *Elm1* expression on tumorigenicity. All clones were tumorigenic, but the incidence of tumor formation was decreased in the M-2.*Elm1*.23-1 cells. Moreover, in vivo growth rates of the transfectants became slower in proportion to the increase in the level of *Elm1* expression (Fig. 7 and Table 1).

Two independent experiments were performed to evaluate the metastatic ability of transfectants. The numbers of metastatic colonies of the transfectants became smaller in proportion to the increase in the level of *Elm1* expression

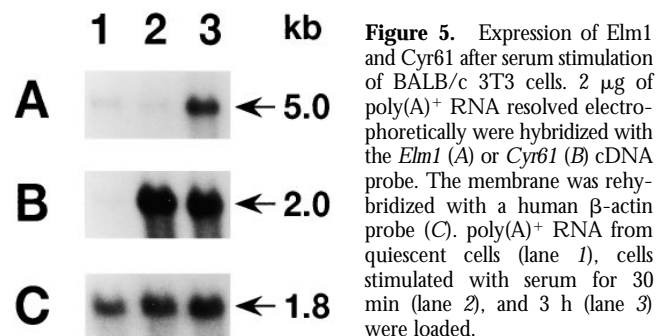


Figure 5. Expression of *Elm1* and *Cyr61* after serum stimulation of BALB/c 3T3 cells. 2 μ g of poly(A)⁺ RNA resolved electrophoretically were hybridized with the *Elm1* (A) or *Cyr61* (B) cDNA probe. The membrane was rehybridized with a human β -actin probe (C). poly(A)⁺ RNA from quiescent cells (lane 1), cells stimulated with serum for 30 min (lane 2), and 3 h (lane 3) were loaded.

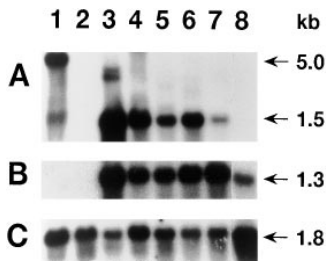


Figure 6. Northern blot analysis of Elm1 transfectants using the Elm1 (A), Neo (B), and β -actin (C) probes. The size of exogenous and endogenous Elm1 transcripts are 1.5 and 5.0 kb, respectively. Lane 1, K-1735 C-23; lane 2, K-1735 M-2; lane 3, M-2.Elm1.23-1; lane 4, M-2.Elm1.20-3; lane 5, M-2.Elm1.0-1; lane 6, M-2.Elm1.6-2; lane 7, M-2.Elm1.20-2; lane 8, M-2.Elm1.8-3.

(Table 2). In particular, the numbers of metastatic colonies of M-2.Elm1.23-1 cells were significantly lower than those of K-1735 M-2 cells in both experiments (Table 2). The numbers of metastatic colonies of M-2.Elm1.20-3 cells were significantly lower than those of K-1735 M-2 cells in experiment 1. Low-metastatic C-23 cells did not produce lung colonies. Metastatic colonies of M-2, M-2.Elm1.8-3, M-2.Elm1.20-3, and M-2.Elm1.23-1 cells in experiment 1 are shown in Fig. 8.

Discussion

We determined the full-length cDNA structure of the *Elm1* gene that was expressed in low-metastatic but not in high-metastatic murine melanoma cells. Elm1 is a novel mouse gene and showed a sequence similarity to the CCN family. CCN family members have been defined as proteins with a mosaic structure because they all contain several motifs shared by various functionally unrelated proteins (7). The *Elm1* gene product highly conserved these motifs, including the IBP-like, VWC, TSP1, and CT domains (Fig. 2). Identity of amino acid sequence between the de-

duced Elm1 and the other CCN family proteins was ~40%, and a dendrogram indicated that no recorded CCN family members would be an orthologue of Elm1. These results suggest that the *Elm1* gene belongs to a new subclass of the CCN family.

CCN family members are considered to function in a wide variety of biological processes, such as tissue regeneration, tumor formation, and embryonic development, as signal molecules to the extracellular matrix (7, 13, 14). CTGF is induced by TGF- β and mediates mitogenic effects of TGF- β on fibroblasts (13). Cyr61 is transcriptionally activated by serum growth factors in fibroblasts, secreted to the extracellular matrix, and enhances the mitogenic effect of basic fibroblast growth factor (bFGF) on fibroblasts and endothelial cells (10, 14). Nov was isolated from an integration site of myeloblastomavirus-associated virus (MAV) and overexpressed in MAV-induced nephroblastoma (9). CTGF and Cyr61 are classified as immediate early genes involved in the control of cell proliferation because they are induced within 30 min after serum stimulation. (10, 12). In contrast to CTGF and Cyr61, Nov is downregulated in proliferating fibroblasts (24). Elm1 was induced by serum, but not induced within 30 min. Thus, Elm1 would play a different role from the other CCN family members in proliferating fibroblasts. CTGF is a mitogenic growth factor (8). Cyr61 itself does not act as a mitogen, but has the ability to enhance the effect of bFGF on DNA synthesis (14). Nov protein has a growth suppressor activity in fibroblasts (9). To examine whether Elm1 has such a growth regulatory function, we performed thymidine incorporation assay using sample mediums of Elm1-transfected K-1735 M-2 cells. However, we could not detect mitogenic activity of Elm1 by itself or the ability of Elm1 to enhance the effect of bFGF on NIH3T3 cells (data not shown). Thus, it is un-

Table 1. *In Vitro* Growth Properties and Tumorigenicity of K-1735 M-2 Cells Transfected with the *Elm1* Gene

Cell line	Expression of Elm1*	Population doubling time [†]	Saturation density in media with 10% FCS	Tumorigenicity [§]	
				Expt. 1	Expt. 2
		<i>h</i>			
K-1735 M-2	0.0	13.8	1.1×10^6	5/5 (21)	5/5 (22)
M-2.Elm1.8-3	0.0	13.1	0.9×10^6	5/5 (20)	5/5 (19)
M-2.Elm1.20-2	0.2	13.5	0.8×10^6	4/5 (27)	5/5 (21)
M-2.Elm1.0-1	0.6	15.0	0.9×10^6	3/5 (32)	5/5 (24)
M-2.Elm1.6-2	1.4	13.3	1.1×10^6	5/5 (37)	5/5 (28)
M-2.Elm1.20-3	1.6	16.1	0.5×10^6	4/5 (>60)	5/5 (>60)
M-2.Elm1.23-1	6.3	16.0	0.3×10^6	2/5 (46)	1/5 (>60)
K-1735 C-23	1.0	13.5	0.9×10^6	0/5	1/5 (49)

*The amount of Elm1 and β -actin mRNA was quantified by Northern blot densitometric analysis of the autoradiogram. RNA loading levels were standardized with respect to the amount of β -actin mRNA. Levels of standardized Elm1 mRNA were expressed relative to the standardized level in K-1735 C-23 cells.

[†]Growth rate was estimated from the logarithmic phase of the growth curve.

[§]Observed for up to 60 d after inoculation of 10^6 viable cells into 5-wk-old syngeneic mice. Tumorigenicity is expressed by the number of mice with tumors per the number of mice injected. Average days of tumors that reached 1 cm³ in each group are shown in parentheses.

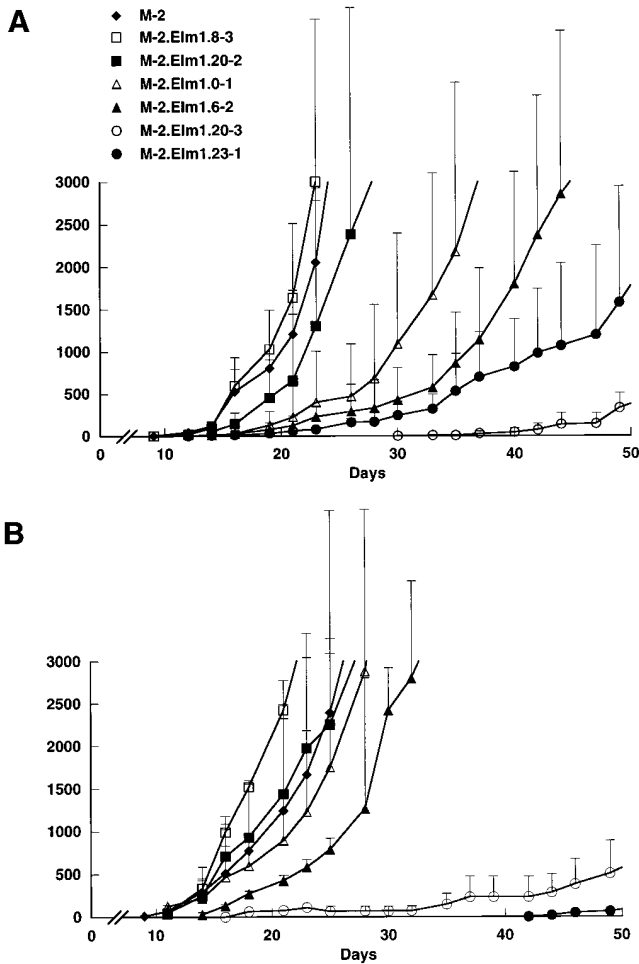


Figure 7. In vivo growth of K-1735 M-2 cells transfected with the *Elm1* gene. (A) Experiment 1; (B) experiment 2. 10^6 cells were inoculated subcutaneously into syngeneic mice and tumor growth was monitored three times per week. Average volume in mice with tumors and SD of values (bars) are shown.

likely that *Elm1* itself has growth regulatory function on the NIH3T3 fibroblast. However, it is possible that *Elm1* might enhance the mitogenic effect of growth factors other than bFGF.

We found that *Elm1* gene transfection resulted in an inhibition of in vivo growth and metastasis formation. Expression of *Elm1* had little suppressive effect on in vitro growth, but a marked suppressive effect on in vivo growth. Suppressive effect of *Elm1* on metastatic potential was associated with suppressive effect of *Elm1* on in vivo growth. In M-2.Elm1.23-1 and M-2.Elm1.20-3 cells that express high levels of *Elm1*, a reduction in the incidence of subcutaneous tumor formation as well as metastatic formation was observed. The reason why the cells failed to form tumors is presently unknown, but may result from alterations in tumor cell–host interactions, such as angiogenesis and/or responses to stimulatory and suppressive cytokines (25). TSP1 inhibits angiogenesis and modulates endothelial cell adhesion, motility, and growth. The antiproliferative activity of

Table 2. Experimental Lung Metastasis of K-1735 M-2 Cells Transfected with the *Elm1* Gene

Cell line*	Lung metastasis		
	Incidence	Median	Range
Expt. 1			
K-1735 M-2	5/5	126	36–162
M-2.Elm1.8-3	5/5	149	143–176
M-2.Elm1.20-2	4/5	115	0–148
M-2.Elm1.0-1	5/5	71	27–127
M-2.Elm1.6-2	3/5	28	0–136
M-2.Elm1.20-3	5/5	3 [‡]	2–7
M-2.Elm1.23-1	0/5	0 [‡]	0
K-1735 C-23	0/5	0 [‡]	0
Expt. 2			
K-1735 M-2	5/5	94	10–153
M-2.Elm1.8-3	5/5	123	34–156
M-2.Elm1.20-2	4/5	66	0–142
M-2.Elm1.0-1	3/5	10	0–100
M-2.Elm1.6-2	5/5	5	3–116
M-2.Elm1.20-3	3/5	8	0–11
M-2.Elm1.23-1	3/5	1 [‡]	0–7
K-1735 C-23	0/5	0 [‡]	0

*Syngeneic mice were injected intravenously with 10^5 viable cells and killed after 22 (experiment 1) and 23 d (experiment 2) of injection. The number of lung colonies was determined using a magnifying glass.

[‡]The number of lung colonies of these cells were significantly different from that of K-1735 M-2 cells (Mann-Whitney U test, $P < 0.01$).

TSP1 is mimicked by a synthetic peptide derived from the type 1 repeats of TSP1 that antagonizes fibroblast growth factor and induces programmed cell death in bovine aortic endothelial cells (26). Thus, it is possible that an inhibition of angiogenesis by the TSP1 domain of *Elm1* leads to the suppression of in vivo growth. Further studies are now in progress on this subject.

Elm1 was isolated as a gene differentially expressed between high- and low-metastatic K-1735 mouse melanoma cells. In K-1735 systems, actin organization, cell adhesion, motility, and a growth rate at a subcutaneous site have been shown to be different between high- and low-metastatic cells (27–29). *Elm1* could be a gene that is involved in the growth of K-1735 cells in vivo. It is noted that the *Elm1* gene is one of several genes involved in the regulation of metastatic potential in K-1735 cells since it was previously shown that expression of the inducible nitric oxide synthase and nm23 genes suppresses tumorigenicity and metastasis of K-1735 cells (25, 30). These sets of genes would cooperatively affect the metastatic potential of K-1735 cells. A more detailed characterization of the *Elm1* gene should allow a critical analysis of the molecular mechanism of metastasis in K-1735 cells.

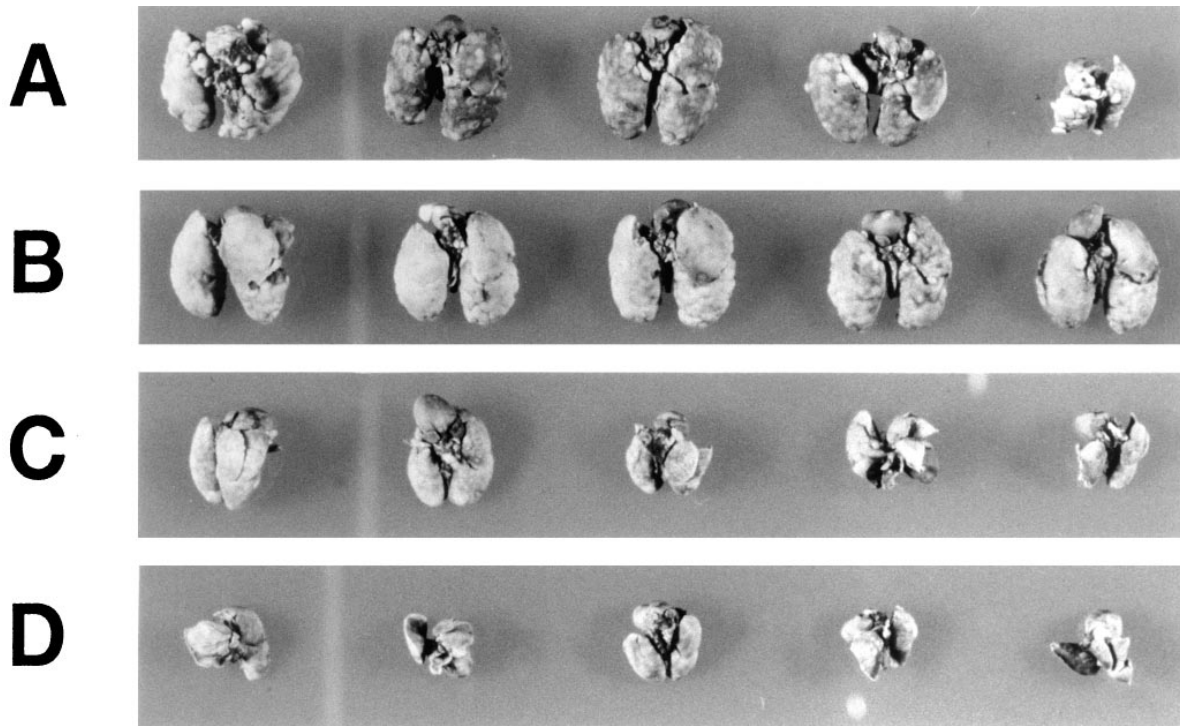


Figure 8. Metastatic potential of K-1735 M-2 cells transfected with the *Elm1* gene. Syngeneic mice were injected intravenously with 10^5 cells and killed 23 d after injection. A, K-1735 M-2; B, M-2.Elm1.8-3; C, M-2.Elm1.20-3; D, M-2.Elm1.23-1.

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