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# Involvement of S100-related Calcium-binding Protein pEL98 (or *mts1*) in Cell Motility and Tumor Cell Invasion

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**We examined the relationship between cell motility and the expressions of pEL98 (*mts1*) mRNA and protein in various murine normal and transformed cells. The expression of pEL98 (*mts1*) in v-Ha-*ras*-transformed NIH3T3 cells and in normal rat kidney cells transformed by either v-Ha-*ras* or v-*src* was increased over that in the corresponding parental cells at both mRNA and protein levels. The expression in normal rat fibroblasts (3Y1) transformed by v-Ha-*ras* was also increased compared with that in 3Y1 cells. However, the expression of pEL98 (*mts1*) in 3Y1 cells transformed by v-*src* was increased in one clone (*src* 3Y1-K), but decreased in another clone (*src* 3Y1-H). The expression level of pEL98 (*mts1*) correlated well with cell motility, which was examined by measuring cell tracks by phagokinesis. In order to test direct involvement of the pEL98 (*mts1*) protein in cell motility, *src* 3Y1-H cells that showed low cell motility were transfected with pEL98 cDNA. The transfectants expressing large amounts of the pEL98 protein showed significantly higher cell motility than *src* 3Y1-H cells. The expression of pEL98 (*mts1*) was also found to be correlated with motile and invasive abilities in various clones derived from Lewis lung carcinoma. These results suggest that the pEL98 (*mts1*) protein plays a role in regulating cell motility and tumor cell invasiveness.**

Key words: pEL98 — *mts1* — Calcium-binding protein — Motility — Invasion

Cell motility is essential for many biological phenomena such as inflammatory reactions, tissue repair and tumor cell invasion and metastasis.[1,2] Many substances including complement-derived factors,[3] formyl peptides,[4] autocrine motility factor,[1] histamine[5] and extracellular matrix components[6,7] are reported to stimulate motility in normal and tumor cells *in vitro*. Recognitions by the specific cell surface receptors of these substances followed by transduction of this information into intracellular signals and regulation of the actin cytoskeleton (actomyosin system) may be required to elicit motility.[8] Although signal transduction pathways that mediate cell motility are poorly understood, several molecules are implicated, including *rho* p21, a *ras*-related small GTP-binding protein,[9] pertussis toxin-sensitive GTP-binding proteins,[6,10–12] and nm23/NDP kinase.[13] In addition to these molecules, evidence is accumulating that $Ca^{2+}$ plays a role as a second messenger for stimulating chemotaxis in eukaryotic cells.[8,14–18]

Although the biochemical mechanisms that transduce $Ca^{2+}$ signals downstream to cellular motile apparatus are largely unknown, $Ca^{2+}$-binding proteins may be involved. Calmodulin, S100 protein and S100-related proteins, all of which have an EF-hand $Ca^{2+}$-binding motif, are candidates. $Ca^{2+}$-Calmodulin reverses the inhibition of actomyosin ATPase activity by caldesmon, a calmodulin- and F-actin-binding protein.[19] S100 protein inhibits the binding of caldesmon to F-actin, and reverses the inhibitory action of caldesmon on skeletal muscle actomyosin ATPase activity.[20] Although little is known about the functions and effector proteins of S100-related proteins, they may also participate in regulation of the cytoskeleton. It has been reported that p11 associates with components of the cytoskeleton in fibroblasts.[21] Furthermore, we have recently found that pEL98, a cDNA of which was initially identified as a marker distinguishing established cell lines from the primary culture,[22] binds to tropomyosin, one of the F-actin-binding proteins, in a $Ca^{2+}$-dependent manner.[23]

Ebralidze *et al.* have isolated the gene *mts1*, which is specifically expressed in metastatic tumor cells, and found that it is identical to pEL98.[24] Davies *et al.* have recently reported that transfection of a benign rat mammary epithelial cell line with the gene for p9Ka, the rat homologue of the gene product of pEL98 or *mts1*, results in induction of metastatic phenotype.[25] Although the precise mechanism(s) by which *mts1* or p9Ka regulates metastatic phenotype of tumor cells is unclear, it is possible that these S100-related proteins interact with components of the cytoskeleton and thereby modulate cell motility and invasion.

In the present study, we examined whether the expression level of pEL98 (*mts1*) is correlated with cell motility and also with tumor cell invasion. We report here that the expression level of pEL98 (*mts1*) is correlated well with motile ability in various normal cell lines and their

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transformants with either v-*ras* or v-*src*. We also demonstrate that the expression level of the pEL98 (*mts1*) protein is correlated with motile and invasive abilities in various clones derived from Lewis lung carcinoma.

## MATERIALS AND METHODS

**Cells and culture** NIH3T3 cells and those transformed by v-Ha-*ras* (pH1-3) were kindly provided by Dr. T. Sekiya (National Cancer Center Research Institute, Tokyo). Normal rat fibroblast 3Y1 cells, 3Y1 cells transformed by v-*src* (named temporarily *src* 3Y1-H),[26] normal rat kidney (NRK) cells, and NRK cells transformed by either v-Ha-*ras* (*ras* NRK) or v-*src* (*src* NRK) were gifts from Dr. H. Sakiyama (National Institute of Radiological Sciences, Chiba). 3Y1 cells transformed by v-Ha-*ras* (*ras* 3Y1) were obtained from the Japanese Cancer Research Resources Bank. 3Y1 cells transformed by v-*src* (named temporarily *src* 3Y1-K) were established by infection of the 3Y1-B clone 1-6 line[27] with Schmidt-Ruppin D strain Rous sarcoma virus[28] and obtained from Dr. S. Taniguchi (Kyushu University, Fukuoka). Clones derived from Lewis lung carcinoma were established in our laboratory and some of them were characterized previously.[29-32] NIH3T3 and pH1-3 cells were cultured in Eagle's minimum essential medium supplemented with 10% heat-inactivated (56°C, 30 min) calf serum, 100 $\mu$g/ml of streptomycin and 100 units/ml of penicillin. Other cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum and antibiotics. They were grown in a humidified atmosphere of 5% $CO_2$ in air at 37°C.

**RNA extraction and Northern blot analysis** Total RNA was extracted with guanidinium thiocyanate.[33] Ten $\mu$g of total RNA was electrophoresed on 1% agarose gel containing formaldehyde[33] and transferred to nylon filters. Blots were hybridized with a $^{32}$P-labeled pEL98 DNA probe which was prepared by the random primer method.[33] The sequence of pEL98 cDNA has been described elsewhere.[22] Filters were finally washed at 50°C in 30 m*M* NaCl, 3 m*M* sodium citrate and 0.1% sodium dodecyl sulfate (SDS).

**Antibody production** Anti-pEL98 antiserum was produced by immunizing a New Zealand White female rabbit with purified recombinant pEL98 protein. Preparation of the recombinant pEL98 protein and the immunizing protocol are described elsewhere.[23] The specificity of the antiserum was confirmed by Western blot analysis.

**Preparation of cell extract** Subconfluent cells were washed three times with cold Dulbecco's phosphate-buffered saline. They were scraped off the culture dishes with a rubber policeman and pelleted by centrifugation. They were then lysed with a cold extraction buffer consisting of 1% (v/v) Triton X-100, 150 m*M* NaCl, 50 m*M* Tris-HCl, pH 7.5, 1 m*M* EGTA and 1 m*M* PMSF for 10 min on ice. The resulting cell extract containing soluble cellular proteins was centrifuged at 10,000*g* for 10 min and the supernatant was used immediately or stored frozen. Almost all of the pEL98 (*mts1*) proteins could be recovered in Triton-soluble fractions by this procedure.[23]

**SDS-polyacrylamide gel electrophoresis and Western blot analysis** SDS-polyacrylamide gel electrophoresis was performed as described previously.[34] Protein samples were electrophoresed on 15% polyacrylamide gels under reducing conditions. The resolved proteins were electrophoretically transferred to nitrocellulose membrane[35] and the pEL98 (*mts1*) protein was detected using anti-pEL98 antibodies and an ECL Western blotting detection kit (Amersham).

**Expression construct of pEL98 cDNA and transfection** The coding sequence of pEL98 cDNA[22] was excised from pGEM-3Z (Promega Corp.) using *Pst* I and *Kpn* I. The fragments were blunt-ended with T4 DNA polymerase, ligated to the *Bam* HI linker, and cleaved with *Bam* HI. The fragments were then cloned into a *Bam* HI-cut pMEXneo (kindly provided by Dr. M. Barbacid, Bristol-Myers Squibb Pharmaceutical Research Institute)[36] to form a plasmid pMEXneo/pEL98. Competent *Escherichia coli* JM109 cells were transformed with the pMEXneo/pEL98 and recombinant clones were screened by restriction enzyme analysis. *src* 3Y3-H cells were transfected with the plasmid pMEXneo/pEL98 using a lipofectin method. Stable transfectants were then selected by exposure to 400 $\mu$g/ml of geneticin (G418, Gibco), and screened for pEL98 protein expression.

**Cell motility assay** Cover slips were coated with 10 $\mu$g/ml of human plasma fibronectin (Gibco) overnight at 4°C. These cover slips were further coated with colloidal gold particles as described previously[37] and thoroughly rinsed with DMEM. Cells were seeded onto these cover slips at a density of $2 \times 10^3$ cells per cover slip in 2 ml of DMEM containing 10% fetal bovine serum and cultured for 18 h or 21 h. The cells were fixed with formaldehyde and then the cover slips were mounted. The area where cells had moved and phagocytized the colloidal gold particles was delineated after enlarging it by a microprojector (Nikon). The areas were digitized and measured by using an image analysis program (NIH Image). Cell motility was evaluated by measuring more than thirty areas free of the gold particles.

**Invasion assay** Invasive ability was measured as described previously[38] with some modifications. Briefly, polycarbonate filters, 8-$\mu$m pore size (Costar), were coated with an extract of basement membrane components (Matrigel; 30 $\mu$g/filter; Collaborative Research Co.), dried, and reconstituted with DMEM. The coated filters were placed in the blind well Boyden chambers.

The cells to be tested were collected by short exposure to 2 m*M* EDTA, resuspended in DMEM containing 0.1% bovine serum albumin, and incubated for 20 min at 37°C. The cells ($2\times10^5$) were then placed in the upper compartment of the chamber. NIH3T3-conditioned media were used as chemoattractants in the lower compartment of the chamber. After incubation for 5 h at 37°C, the filters were removed and the cells on the upper surface of the filter were completely removed by wiping with a cotton swab. The filters were fixed and stained with May-Gruenwald-Giemsa. Cells from various areas (at least 10 areas) of the lower surface were counted.

**Protein determination** Protein concentration was determined by the method of Bradford[39] using bovine serum albumin as a standard.

## RESULTS

**Expression of pEL98 (*mts1*) mRNA and protein in various normal and v-Ha-*ras*- and v-*src*-transformed cells** The expression of pEL98 (*mts1*) mRNA in NIH3T3, v-Ha-*ras*-transformed NIH3T3 (pH1-3), NRK, v-Ha-*ras*-transformed NRK (*ras* NRK), v-*src*-transformed NRK (*src* NRK), 3Y1, v-Ha-*ras*-transformed 3Y1 (*ras* 3Y1) and two clones derived from v-*src*-transformed 3Y1 cells (named temporarily *src* 3Y1-H and *src* 3Y1-K) was examined by Northern blot analysis (Fig. 1). As reported previously,[22] the probe hybridized to mRNA of approximately 0.6-kilobase in size. The expression of pEL98 (*mts1*) mRNA in pH1-3 cells was increased over that in NIH3T3 cells (lanes 1 and 2). Similarly, the expression of pEL98 (*mts1*) mRNA in *ras* NRK and *src* NRK cells was more pronounced than in NRK cells (lanes 3–5). The level of pEL98 (*mts1*) mRNA in *ras* 3Y1 cells was also increased over that in 3Y1 cells (lanes 6 and 7). In the case of *src* 3Y1 cells, however, conflicting results were obtained; that is, the expression of pEL98 (*mts1*) mRNA in *src* 3Y1-K was increased compared with that in 3Y1 cells (lanes 6 and 9), whereas it was decreased in *src* 3Y1-H cells (lanes 6 and 8).

Western blot analysis was also carried out to measure the expression level of the pEL98 (*mts1*) protein in these cells (Fig. 2). Reflecting the expression at the RNA level, the amounts of the pEL98 (*mts1*) protein in pH1-3, *ras* NRK, *src* NRK, *ras* 3Y1 and *src* 3Y1-K cells were more abundant than those in the corresponding parental cells.

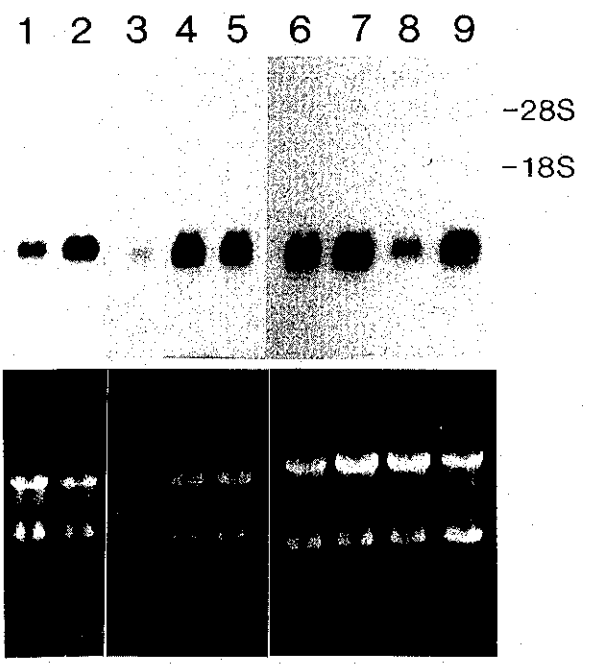


Fig. 1. Northern blot analysis of pEL98 mRNA in various normal and transformed cells. Total RNA from various cells were electrophoresed on 1% agarose gels containing formaldehyde, transferred onto nylon filters, and hybridized with a $^{32}$P-labeled pEL98 probe. Lane 1, NIH3T3; lane 2, pH1-3; lane 3, NRK; lane 4, *ras* NRK; lane 5, *src* NRK; lane 6, 3Y1; lane 7, *ras* 3Y1; lane 8, *src* 3Y1-H; lane 9, *src* 3Y1-K. Ethidium bromide staining of the agarose gels is shown at the bottom of the figure.

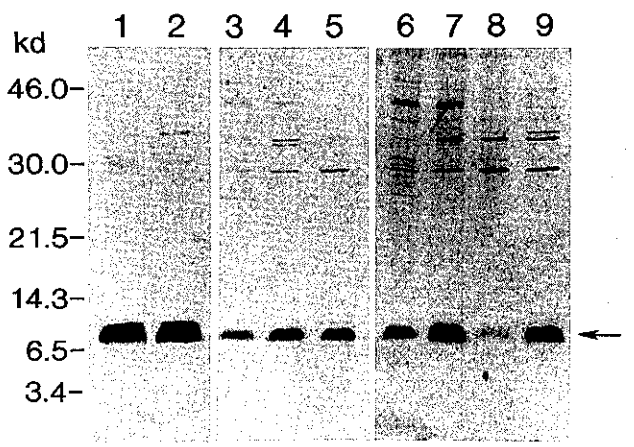


Fig. 2. Western blot analysis of the pEL98 protein in various normal and transformed cells. Cell extracts (20 $\mu$g of total protein) prepared by extraction with buffer containing EGTA were electrophoresed on 15% acrylamide gels under reducing conditions and the resolved proteins were transferred electrophoretically to nitrocellulose membrane. The pEL98 protein was detected using polyclonal anti-pEL98 antibodies and an ECL Western blotting detection kit. Lane 1, NIH3T3; lane 2, pH1-3; lane 3, NRK; lane 4, *ras* NRK; lane 5, *src* NRK; lane 6, 3Y1; lane 7, *ras* 3Y1; lane 8, *src* 3Y1-H; lane 9, *src* 3Y1-K. The arrow indicates the position of the pEL98 protein, whose molecular mass is approximately 10 kDa.[23] The bands in the upper portion of the gels are nonspecific, because these bands could also be detected when preimmune serum was used (data not shown).

The amount of the pEL98 (*mts1*) protein in *src* 3Y1-H cells was less than that in 3Y1 cells.

**Correlation between pEL98 (*mts1*) expression and cell motility** In order to examine the correlation between pEL98 (*mts1*) expression and cell motility, we measured the motile abilities of the cell lines described above. The results showed that pH1-3 cells were more motile than NIH3T3 cells (Fig. 3A). *ras* NRK and *src* NRK cells showed higher cell motility than NRK cells (Fig. 3B). *ras* 3Y1 and *src* 3Y1-K cells were also more motile than 3Y1 cells, whereas *src*3Y1-H cells were less motile than 3Y1 cells (Fig. 3C). When bovine serum albumin-coated cover slips were used instead of fibronectin-coated ones, essentially the same results were obtained, although the cells were less motile on bovine serum albumin-coated cover slips than on fibronectin-coated cover slips (data not shown). Therefore, these results suggest that there is a positive correlation between the expression level of pEL98 (*mts1*) and cell motility.

**Increased cell motility in *src* 3Y1-H cells transfected with pEL98 cDNA** In order to obtain more direct evidence that the pEL98 (*mts1*) protein is involved in regulation of cell motility, doubly cloned *src* 3Y1-H cells were transfected with pEL98 cDNA and clones expressing larger amounts of the pEL98 protein than the parental cells were isolated. These transfectants included EL1, EL2, EL3 and EL4. Transfected clones with the neomycin-resistance gene but without pEL98 cDNA were also isolated as controls. These were V1, V2 and V3. Fig. 4A shows an immunoblot analysis of the expression of the pEL98 protein in the parent and transfectants. All the pEL98 cDNA-transfected clones showed higher expression of the pEL98 protein than *src* 3Y1-H cells and control clones, but lower than 3Y1 cells. Isolation of clones expressing much higher amounts of the pEL98 protein was unsuccessful. Cell motility was evaluated in these transfectants and the results are shown in Fig. 4B. All the pEL98 cDNA-transfected clones showed significantly higher cell motility than *src* 3Y1-H cells and control clones, but lower than 3Y1 cells. These results indicate that the pEL98 (*mts1*) protein is involved in regulating cell motility. No significant alteration in cell growth or cell morphology was observed in these transfectants (data not shown).

**Correlation between pEL98 (*mts1*) expression in clones derived from Lewis lung carcinoma and their motile and invasive abilities** Cell motility is essential for tumor cells to invade surrounding tissues and metastasize to distant organs. We therefore examined the correlation between pEL98 (*mts1*) expression and cell motility as well as invasive ability in various clones (C2, D6, A11, P29 and P34) derived from Lewis lung carcinoma. Fig. 5A and B show the results of Northern and Western blot analyses of the expression of pEL98 (*mts1*) in these clones, respectively. The lanes of Western blot were scanned with a laser densitometer and the relative expression level of

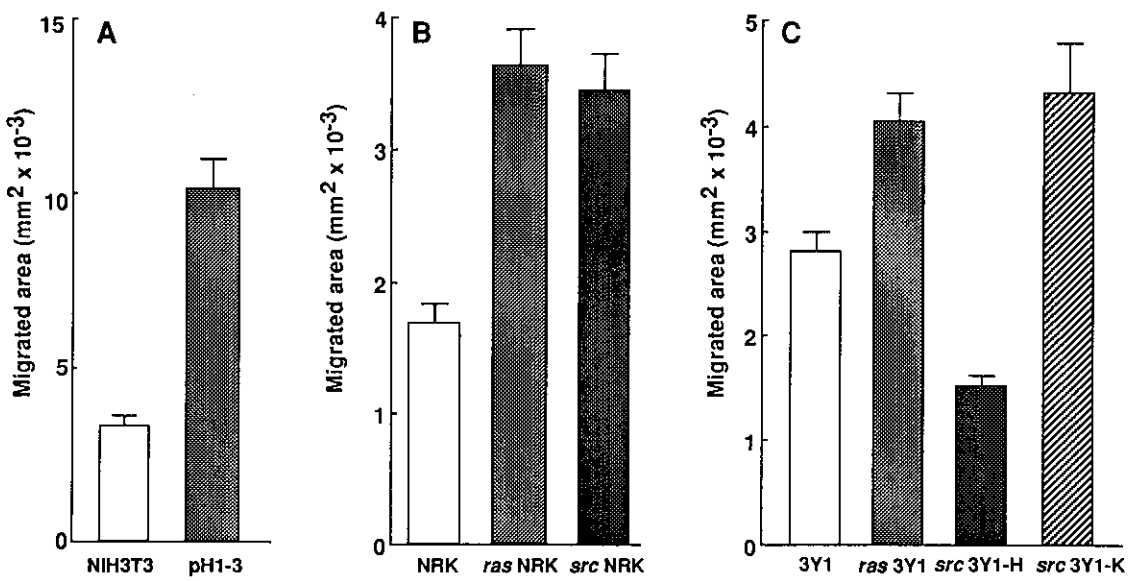


Fig. 3. Cell motility of various normal and transformed cells on fibronectin- and colloidal gold-coated cover slips. Cells were seeded onto a homogeneous layer of gold particles prepared on fibronectin-coated cover slips and cultured for 21 h. The areas free of gold particles were measured. The values shown are the mean±standard error of at least 30 areas. (A) Migration of NIH3T3 and pH1-3 cells. (B) Migration of NRK, *ras* NRK and *src* NRK cells. (C) Migration of 3Y1, *ras* 3Y1, *src* 3Y1-H and *src* 3Y1-K cells.

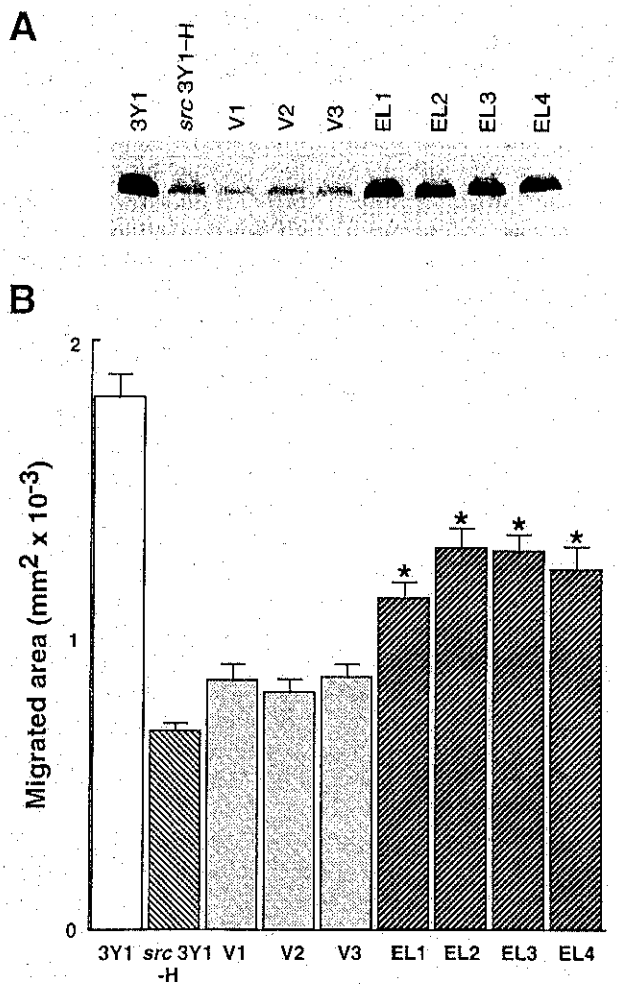


Fig. 4. Expression of the pEL98 protein in *src* 3Y1-H cells transfected with pEL98 cDNA and their motility. (A) Immunoblot analysis of the pEL98 protein. Cell extracts (20 $\mu$g of total proteins) prepared by extraction with buffer containing EGTA were subjected to immunoblot analysis. Only the region of interest is shown. (B) Cell motility. Cell motility was measured as described in the legend to Fig. 3. EL1, EL2, EL3 and EL4 represent clones transfected with pEL98 cDNA. V1, V2 and V3 represent clones transfected with the expression vector alone. The values shown are the mean $\pm$ SE of at least 50 areas. $*$, The difference is significant by Student's $t$ test at $P<0.001$, as compared to migration of control clones.

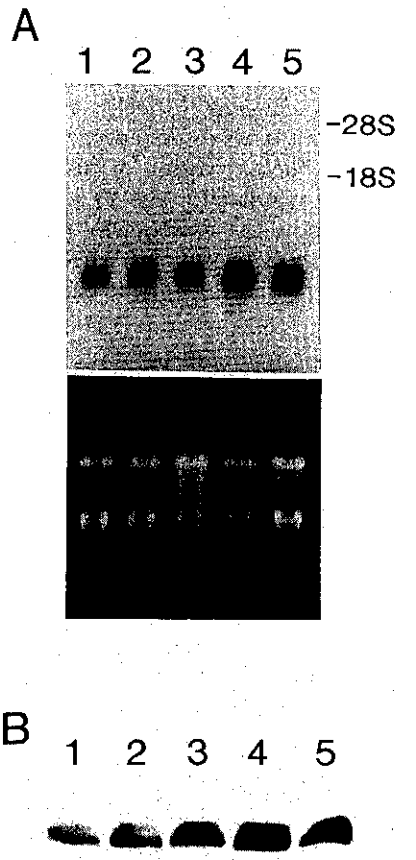


Fig. 5. Expressions of the pEL98 mRNA and protein in various clones derived from Lewis lung carcinoma. (A) Northern blot analysis of pEL98 mRNA. Equivalent amounts of total RNA (10 $\mu$g) were subjected to Northern blot analysis. Ethidium bromide staining of the gel is also shown. (B) Immunoblot analysis of the pEL98 protein. Cell extracts (20 $\mu$g of total protein) prepared by extraction with buffer containing EGTA were subjected to immunoblot analysis. Only the region of interest is shown. Lane 1, C2; lane 2, D6; lane 3, A11; lane 4, P29; lane 5, P34.

the pEL98 protein in each clone is presented in Table I. Motile ability and *in vitro* invasive ability of these clones were evaluated and the results are also shown in Table I. P29 and P34 cells, which expressed greater amounts of the pEL98 (*mts1*) mRNA and protein than C2, D6 and A11 cells, were more motile than the other clones. The motile abilities (migrated areas) of the clones are plotted against the relative expression levels of the pEL98 protein in Fig. 6. The results showed a significant positive correlation between the expression level of the pEL98 protein and the motile ability (Fig. 6A; $r=0.903$, $P<0.05$). Similarly, P29 and P34 cells were more invasive than the other clones and a positive correlation was observed between the expression level of the pEL98 protein and the invasive ability (Fig. 6B; $r=0.947$, $P<0.02$).

## DISCUSSION

We investigated the expression of pEL98 (*mts1*) in various normal and v-*ras*- and v-*src*-transformed cells at both mRNA and protein levels. The results showed that

the expression of pEL98 (*mts1*) was increased in pH1-3 cells over that in NIH3T3 cells. Moreover, the expression levels of the pEL98 (*mts1*) mRNA and protein were higher in *ras* NRK cells, *ras* 3Y1 cells, *src* NRK cells and *src* 3Y1-K cells than those in the corresponding parental cells. In contrast, the expression of pEL98 (*mts1*) was significantly down-regulated in *src* 3Y1-H cells. At present, we do not know the reason why pEL98 (*mts1*) is expressed differently between *src* 3Y1-K and *src* 3Y1-H cells, but several possibilities might explain this apparently conflicting result. First, the integration site of the *src* gene may be different between these two cell lines and as a result the expression of pEL98 (*mts1*) is altered differently. Second, the expression of pEL98 (*mts1*) may not be under the control of the *src* gene product. Third, the expression of pEL98 (*mts1*) may be different in different clones, merely reflecting clonal heterogeneity. In any case, these results indicate that although tumor cells tend to show higher expression of pEL98 (*mts1*) than normal cells there is no consistent relation between pEL98 (*mts1*) expression and transformation. In fact, both cell lines were highly tumorigenic when they were injected in nude mice (data not shown).

Table I. Expression Level of the pEL98 Protein and Motile and Invasive Abilities of Various Clones Derived from Lewis Lung Carcinoma

| Clone | Expression level of pEL98 protein (arbitrary unit)[a] | Migrated area ($mm^2 \times 10^{-3}$)[b] | Invasive ability (No. of cells invaded/field)[c] |
|---|---|---|---|
| C2 | 1.00 | 3.18±0.28 | 75.5±19.8 |
| D6 | 1.32 | 5.25±0.39 | 80.9±14.2 |
| A11 | 1.87 | 3.39±0.25 | 107.3±17.7 |
| P29 | 2.85 | 12.70±0.92 | 144.0±11.7 |
| P34 | 2.63 | 11.97±0.89 | 157.9±15.5 |

*a*) The lanes in Fig. 5B were scanned with a laser densitometer and the relative amount of the pEL98 protein expressed in each clone was calculated.
*b*) More than 30 areas free of gold particles were measured. Mean±SE.
*c*) The number of invaded cells in at least 10 areas was counted. Mean±SE.

Previously, the expression of pEL98 was suggested to be related to "immortalization" and/or cell growth.[22,40] However, since mortal cells such as lymphocytes and macrophages express pEL98 (*mts1*) (see below), it may not be directly involved in "immortalization." Concerning the role of pEL98 (*mts1*) in regulating cell growth, comparison of the growth potential of the two v-*src* transformed 3Y1 cell lines used in this study might be informative. So, we examined the *in vitro* growth rate and anchorage-independent growth of *src* 3Y1-K and *src* 3Y1-H cells and found that both cell lines showed comparable *in vitro* growth rate and colony formation in agar (data not shown). Therefore, pEL98 (*mts1*) expression may not be directly related to cell growth, although we can not rule out the possibility that a small amount of the pEL98 (*mts1*) protein, as observed in *src* 3Y1-H cells, is sufficient for cells to grow rapidly. Thus, these results

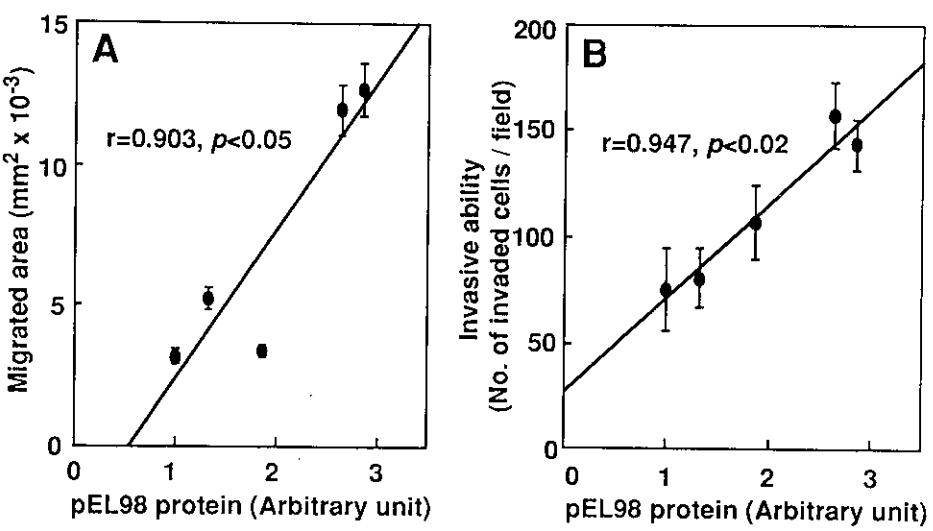


Fig. 6. Relationship between the expression level of the pEL98 protein and the motile and invasive abilities for various clones derived from Lewis lung carcinoma. The motile abilities (migrated area) (A) and invasive abilities (the number of invaded cells/field) (B) of each clone are plotted against the relative expression levels of the pEL98 protein. Values for each clone are presented in Table I.

imply somewhat different function(s) of the pEL98 (*mts1*) protein other than regulation of "immortalization" and/or cell growth.

The present study has demonstrated a positive correlation between the expression level of pEL98 (*mts1*) and cell motility in a variety of normal and v-*ras*- or v-*src*-transformed cell lines. In addition, we observed a significant correlation ($r=0.903$, $P<0.05$) between the expression of the pEL98 (*mts1*) protein and the motile ability in clones derived from Lewis lung carcinoma. Furthermore, transfection of pEL98 cDNA into *src* 3Y1-H cells resulted in enhancement of cell motility. Therefore, it appeared that the pEL98 (*mts1*) protein was involved in regulating cell motility.

Cell motility is required for invasion and metastasis.[41] It has been shown that the gene *mts1* was specifically expressed in metastatic cells, but not in nonmetastatic cells[24] and that p9Ka is able to induce metastatic phenotype in a benign rat mammary epithelial cell line.[25] Although the mechanism(s) by which *mts1* or p9Ka influences the metastatic phenotype of tumor cells is unknown, our present observations imply that increased expression of these proteins results in augmentation of cell motility and thereby invasive ability. Then, we examined the relationship between the expression level of pEL98 (*mts1*) and *in vitro* invasive ability in various clones isolated from Lewis lung carcinoma. The results showed a positive correlation between the expression level of pEL98 (*mts1*) and *in vitro* invasive ability ($r=0.947$, $P<0.02$) and also between the motile ability and the invasive ability ($r=0.899$, $P<0.05$).

How does the pEL98 (*mts1*) protein regulate cell motility? What are the downstream targets of the pEL98 (*mts1*) protein? With regard to these questions, it is of interest to note that the p9Ka protein associates along actin stress fibers in cells transfected with the gene for p9Ka[25] and that, more specifically, the pEL98 (*mts1*) protein binds to tropomyosins in a $Ca^{2+}$-dependent manner.[23] The functions of tropomyosins in microfilaments are poorly understood, but they appear to play a regulatory role in defining actin filament assembly and organization during cell motility, cell division and changes in cell shape.[42] Therefore, if the pEL98 (*mts1*) protein exerts its effects on F-actin-tropomyosin interactions and hence on actomyosin ATPase activity in a $Ca^{2+}$-dependent manner, it is possible that motility could be influenced through these mechanisms. If this is the case, a rise in intracellular calcium ($[Ca^{2+}]_i$) might be necessary for tumor cell motility. Concerning this point, Savarese *et al.* have reported that an increase in $[Ca^{2+}]_i$ is necessary for tumor cell motility.[18] Furthermore, calcium channel blockers are reported to inhibit motility of monocytes and neutrophils.[14, 15] At present, however, we have no evidence indicating that $[Ca^{2+}]_i$ is indeed elevated in the cell lines used in this study during migration. Further studies are needed to clarify the relationships among levels of $[Ca^{2+}]_i$ and the pEL98 (*mts1*) protein and cytoskeletal organization during cell motility.

Expression of the pEL98 (*mts1*) mRNA can be detected in a limited number of normal organs and cells. It is expressed in the so-called "lymphoid organs" such as spleen and thymus and in bone marrow and peripheral blood lymphocytes, but not in brain, liver, heart, lung, kidney, testis and ovary[24, 40] It is of interset to note that we have recently found that pEL98 mRNA is expressed in large amounts in peritoneal macrophages (K. Takenaga *et al.*, unpublished observation). Both lymphocytes and macrophages are known to be highly motile. Therefore, the pEL98 (*mts1*) protein is not ubiquitous, but is probably expressed in certain cell types that show higher cell motility.

Cell motility is regulated by several biochemical pathways and mediators. In addition to $Ca^{2+}$-related pathways, *rho* p21 and its inhibitory GDP/GTP exchange protein (*rho* GDI) are reported to be involved in regulating cell motility, possibly through the actomyosin system.[9] Pertussis toxin-sensitive GTP-binding proteins also play a role in regulating motility.[10–12] Probably, these signal transduction pathways act in concert to influence cell motility and tumor cell invasion. Further studies on such pathways and mediators would undoubtedly give us clues to understand the mechanisms by which cell motility is controlled and to suppress tumor cell invasion and metastasis.

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