〔ORIGINAL〕

Transfection of Cu-Zn Superoxide Dismutase Antisense cDNA Promotes Motility and Metastasis of Murine Fibrosarcoma Cells

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Abstract

Previously I and my coleagues reported that a clone of human tongue cancer cells with higher invasiveness expressed lower Cu-Zn SOD (superoxide dismutase) activities than a clone with low invasiveness and the suppression of Cu-Zn SOD activity by antisense cDNA transfection resulted in enhanced motility of human tongue cancer cells *in vitro*. However, whether or not this inverse relation between intracellular Cu-Zn SOD activity and motility of tumor cells is generally found in other tumor cells and whether the intracellular Cu-Zn SOD in fact defines *in vivo* metastatic potential were undetermined.

In the present study, I transfected antisense Cu-Zn SOD cDNA into murine Meth A sarcoma-derived ML-01 cells with low metastatic property and obtained five clones. Two clones with different SOD activities, ML-AS2 with the most suppressed activity and the ML-AS5 with the least suppressed activity, were analyzed for their motility and metastatic ability. The result was that ML-AS2 exhibited 4 fold increased cell motility and ML-AS5 exhibited 2.2 fold increased motility as compared to the mock transfectant ML-neo cells. In addition, superoxide treatment enhanced the invasiveness of ML-AS clones but not of ML-neo. Metastatic potential of ML-AS2 and ML-AS5 were 4.5 and 2.1 fold of that of ML-neo, respectively.

Thus, these results suggested that the intracellular Cu-Zn SOD level and *in vivo* metastatic potential are inversely related via regulating cell motility.

Key words : motility, metastasis, SOD, superoxide

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Introduction

Metastasis is a multistep process which requires a variety of characteristics for tumor cells to accomplish. Numerous host factors affect the metastatic capability of tumor cell. It was found that excited oxygen species which were derived from tumor cells themselves and surrounding host cells are involved in tumor invasion and metastasis[1-5].

Accordingly, a scavenger of superoxide, superoxide dismutase (SOD) is speculated to be related to tumor invasion and metastasis. In fact, Kwee *et al.*[6] reported that the intracellular Mn SOD activity was low in mouse tumor cells showing high metastatic potential, and high in cells showing low metastatic potential. Safford *et al.*[7] demonstrated that the *in vivo* metastatic potential of mouse fibrosarcoma cells was suppressed when SOD was overexpressed by the transfection of its gene, although the mechanism underlying this effect was not elucidated. Yoshizaki *et al.*[5] demonstrated that the experimental metastasis of mouse fibrosarcoma Meth A cell was suppressed by intravenous administration of recombinant human SOD (rh SOD) as the result of the inhibition of invasiveness. Utilizing human oral squamous carcinoma cells I and my coleagues[8] further clarified the fact that the invasion evoked by superoxide is due to the enhancement of the motility of tumor cells. When the oral carcinoma cells were transfected with antisense SOD, the cells showed enhanced invasiveness associated with increased motility, particularly under the condition of superoxide generation. However, these results were obtained merely by *in vitro* assay employing only one cell line.

Therefore, in the present study, I examined if a similar observation to the one that my coleagues[8] found with human oral cancer cell line could be confirmed with murine tumor cell transfected with the antisense cDNA. In addition, using the transfectants, I attempted to elucidate whether or not the intracellular SOD level and *in vivo* metastatic potential are indeed inversely correlated via regulating cell motility.

Materials and Methods

Cells and Cell Culture

A murine Meth A sarcoma-derived ML-01 cell with low metastatic property and MH-02 cell with high metastatic property were used. In regard to these cells, Mogi *et al.*[9] reported the following experimental results. When 5×10^4 of MH-02 cells were intravenously injected, 182.1±31.5 pulmonary metastatic nodules on the lung surface were formed. On the other hand, ML-01 formed only 40.6±12.5 of pulmonary metastatic nodules. Cells were cultured in RPMI 1640 media (Nissui, Tokyo, Japan) containing 10% FBS, 100 units/ml penicillin G and 100mg/ml streptomycin using culture flasks in humidified 5% CO_2 at 37°C. Cell proliferation was determined by counting viable cells left unstained trypan blue dye staning.

Reagents and *In Vitro* Generation of Superoxide

Catalase, Xanthine oxidase (XOD) and Hypoxanthine (HPX) were obtained from Sigma

Chemicals (St. Louis, MO). Superoxide anion (O_2^-) was generated by co-incubating 7 x 10^{-4} units/ml XOD with 4 μg/m HPX[3].

Antibodies

The anti-rat Cu-Zn SOD rabbit IgG and anti-rat Mn-SOD rabbit IgG were kind gifts from Dr. Naoyuki Taniguchi, Department of Biochemistry, Osaka University School of Medicine, Osaka, Japan.

Determination of Intracellular SOD Activity

The total SOD activity of the whole cell homogenate was assayed spectrophotometrically by the nitroblue tetrazolium (NBT) method, as described by Oberley and Spitz[10]. One unit of SOD activity was defined as the amount of protein in the cell homogenate necessary to decrease the reference rate of NBT reduction to 50% of the maximum inhibition. Protein concentrations in cell homogenate were determined by the Bio-Rad Protein Assay (Richmond, CA). Intracellular SOD activity was expressed in units per mg protein. The Mn SOD activity was measured by the same method except for the pretreatment of cell lysate with 5 mM sodium cyanide, which inhibitis Cu-Zn SOD but not Mn-SOD. The Cu-Zn SOD activity was calculated from the difference between total cellular SOD and Mn SOD acitivity.

Plasmids

A mammalian expression vector, pRc/CMV which contains a CMV promoter and a selectable neomycin resistance gene under the control of SV40 early promoter was purchased from Invitrogen, San Diego, CA. Full-length coding sequences of mouse Cu-Zn SOD were excised by EcoR I and Xba I digestion of pUC118-mSOD, a kind gift from the ASAHI Chemical Co. Ltd. (Shizuoka, Japan),[11] and were inserted into pRc/CMV at the downstream of CMV promoter in antisense orientation to construct an antisense cDNA, pRc-AS-mSOD. The orientation of the cDNA inserts were confirmed by restriction mapping.

DNA Transfection and Clonal Selection

Transfection was performed by the lipofection method[12]. Briefly, 5 $\times$ 10^5 cells plated in a 60-mm dish were washed twice with 3 ml of Opti-MEM I reduced serum medium (GIBCO BRL, Grand Island, NY) and then immersed in 3 ml of the same medium. The cells were cultured for 6 h at 37°C after the addition of 10 μl of the lipofectamine reagent mixture (GIBCO BRL) containing 3 μg of pRc-AS-mSOD or pRc/CMV and then 3 ml of RPMI 1640 medium containing 20% FCS was added. After a further 72 h of culture, G418 was added at a concentration of 300 μg/ml to select G418-resistant clones in a soft-agar. Five transfectants of pRc-AS-mSOD, thus obtained were named ML-AS1-5. Likewise, a ML-Neo clone which was transfected with pRc/CMV was selected.

Detection of the Cu-Zn SOD Antisense and Neomycin Resistance Gene

DNA was extracted from 1 $\times$ 10^7 transfected ML-01 cells using Sepa Gene^R (Sanko Jun-yaku Co., Tokyo, Japan). The Cu-Zn SOD antisense and neomycin resistance gene in the DNA preparations were detected by PCR analysis. The primers for the Cu-Zn SOD antisense

corresponded to sequences located at nucleotide 6-19 (GATGAAAGCGGTGT) and nucleotide 437-450 (CCTGTGGAGTGATT) of the Cu-Zn SOD gene. The primers for the neomycin resistance gene corresponded to sequences located at nucleotide 2121-2140 (ATCTGATCAAGAGACAGGAT) and nucleotide 2518-2537 (GCCGACGTATGCGAACTAGG) of pRc/CMV. PCR was performed for 30 cycles. Each cycle consisted of 94°C for 1 min, 55°C for 2 min and 72°C for 1.5 min. DNA products were separated on agarose gels, stained with ethidium bromide and detected under ultraviolet light.

Western Blotting

Tumor cells were washed with PBS followed by treatment with lysis buffer [10mM Tris (pH7.4), 150mM NaCl, 0.5% Triton-X 100, 0.2 mM Phenylmethylsulfonyl fluoride (PMSF, Sigma)] . The amount of protein was determined in duplicate by Micro BCA Protein Assay (Pierce, Rockford, IL) according to the manufacturer's method. 20 μg of lysate protein and rat liver Mn-SOD were mixed with 2 x SDS sample buffer, heated at 100°C for 5 min, resolved by SDS-PAGE (15%) and electroblotted to Immobilon P membrane (Millipore, Watford, U. K.). The membrane was blocked with PBS-T (PBS plus 0.1% Tween-20, Kanto Chemicals Co., Inc., TOKYO) containing 5% nonfat milk powder and rinsed twice in PBS-T. The filter was incubated with either one of anti-rat Cu-Zn SOD rabbit IgG or anti-rat Mn-SOD rabbit IgG diluted 1 : 500 in PBS-T for 45 min and then gently agitated for 30 min in PBS-T. This agitation was repeated three times with a fresh change of PBS-T each time. Then the filter was soaked in PBS-T containing Protein A : peroxidase (1 : 1000). The peroxidase bound to the antibody was visualized using luminol (ECL ; Amersham, U. K.).

In Vivo Tumor Growth

Female BALB/c mice of 6 weeks of age were purchased from CLEA Japan Inc. (Sendai, Japan). Animals were cared according to the institutional guideline for care and use of laboratory animals. Tumor cells ($1.0 \times 10^6/100\mu$l) were inoculated into the right abdominal flank of BALB/c mice. At day 7 and day 14, tumor dimensions were measured. Tumor volumes were calculated by the formula $0.4 \times ab^2$, where a is the largest and b the smallest size of the two dimension[9].

Experimental Pulmonary Metastasis

Experimental pulmonary metastasis was carried out as described earlier[9]. Tumor cells which were suspended in 0.1ml of RPMI 1640 medium were introduced via the tail vein of BALB/c mice which ware then sacrificed 14 days after the injection. The lungs were removed and the numbers of metastatic nodules on the surface of the lungs were determined under a dissecting microscope.

Metabolic Radiolabeling of Tumor Cells

Tumor cells were metabolically labeled with ^{125}I-deoxyuridine (^{125}I-IUDR, Amersham) as described by Fidler[13]. Briefly, 1×10^5/ml of cells were cultured with 0.1μCi/ml ^{125}I-deoxyuridine in RPMI 1640 medium containing 10% FBS for 24 h. Cells were then washed

three times with RPMI 1640 medium and resuspended in the same medium.

Motility Assay

^{125}I-IUDR labeled tumor cells (1.0×10^5 cells/ml) in RPMI 1640 were placed in the upper chamber of a Transwell® double chamber well, sized 6.5 mm in diameter with 8μm pores (Costar #3422, Cambridge, MA). A conditioned medium obtained by incubating confluent NIH3T3 cells for 24 h in serum free IMDM medium (GIBCO BRL, Grand Island, NY) was poured into the lower chamber of the Transwell® chamber. After incubation for 12 h at 37°C in humidified 5% CO_2, the cells in the lower chamber were lysed with 1N NaOH and radioactivities were counted by a gamma counter. The motility of cells was expressed by the ratio of the counts of cells in lower chamber to that of cells in the upper chamber.

Cell Attachment Assay

Tumor cell adhesiveness to a Transwell® filter made of polycarbonate was assayed by the methods of Tao and Johnson[14]. Briefly, ^{125}I-IUDR labeled tumor cells (1×10^5 cells/100μl) in RPMI 1640 were placed in the upper chamber of a Transwell® double chamber well sized 6.5 mm in diameter with 0.4μm pores (#3413) which restrain tumor cells from migrating through. The compartments of the upper chambers were centrifuged at 200 x g for 5 min in a plate centrifuge machine (Model CD-60R, TOMY SEIKO CO., Tokyo, Japan) to allow tumor cells to attach onto the filter at the bottom of the chamber wells. As soon as the centrifugation finished, the wells were shaken for 10 s at 100 r. p. m. by a Rotary shaker TAIYO R-II (Taiyo Scientific Co., Tokyo, Japan) and non-adherent cells were removed by gentle aspiration. Adherent cells were gently washed three times with RPMI 1640 medium and solubilized in 1ml of 1N NaOH to measure the radioactivity in a gamma counter. The cell attachment was quantified by taking the ratio of the radioactivity of cells attached to the filter in the upper chamber to that of total cells in the upper chamber.

Statistical Analysis

Statistical analyses for comparison of *in vitro* assay were performed by the Student's *t* test. Mann-Whitney test was used for statistical analyses of *in vivo* assays. The criteria for statistical significance was $p<0.05$.

Results

Determination of Intracellular Cu-Zn SOD Activity

The total SOD activity in the high metastatic MH-02 cells was only about 1/4 of that in the low metastatic ML-01 cells. When the total SOD activity was examined in the presence of sodium cyanide that inhibits Cu-Zn SOD but not Mn SOD, no difference of SOD was detected (Fig. 1). These data suggest that the difference were largely due to differences in intracellular Cu-Zn SOD activity. As I and my coleagues reporetd previously the intracelluar Cu-Zn SOD activity of the SAS cells[8], a negative correlation was confirmed between the cell invasiveness and the intracellular Cu-Zn SOD activity in Meth A cells.

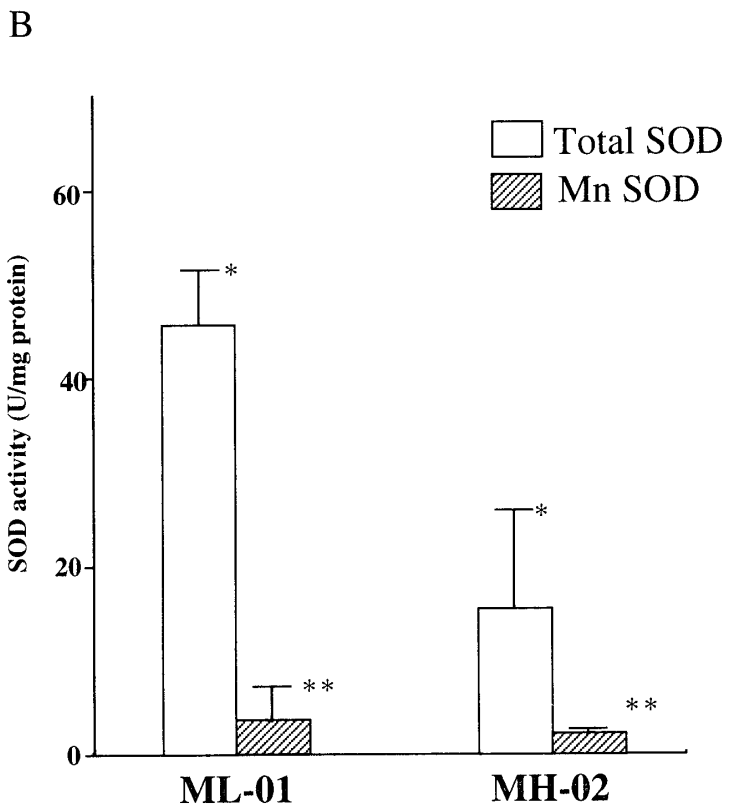

Fig. 1 Intracellular SOD activity of ML-01 and MH-02. The total SOD activity (□) was assayed by the NBT method. Mn SOD (▨) activity was determined in the presense of 5mM sodium cyanide
*, statistically significant ($p<0.01$).
**, statistically not significant (Student's t test)

PCR Analysis for Cu-Zn SOD Antisense cDNA in Transfectants

High-molecular-weight DNA was extracted from antisense transfected ML-AS1-5 clones and the mock transfectant ML-Neo, and was analyzed by the PCR method and subsequent electrophoresis for detecting antisense cDNA sequences (Fig. 2). In all transfectants, a 417-dp band which corresponded to the neo resistance gene of the pRc/CMV vector was detected. The DNA from the ML-AS clones showed an additional 445-bp band that corresponded to the Cu-Zn SOD antisense c-DNA sequence.

Intracelluar SOD Levels of ML-AS Clones and ML-Neo Cells

In order to assure that the Cu-Zn SOD antisense DNA was functioning in the transfected cells, the intracellular SOD activity in the ML-AS clones was assayed. The total SOD activity in all ML-AS clones was lowered to between 50% and 30% of the activity in the ML-Neo cells (Fig. 3). The decrease of the total enzyme activity mainly reflected the reduction of Cu-Zn SOD activity, since sodium cyanide inhibition assay showed no significant difference of Mn-SOD activity between the ML-AS clones and the ML-Neo cells. Among the five ML-AS clones, ML-AS5 showed the least suppression of Cu-Zn SOD activity and ML-AS2 showed the most. I then examined the expression of Cu-Zn SOD protein in the cell lysates of the two ML-AS clones,

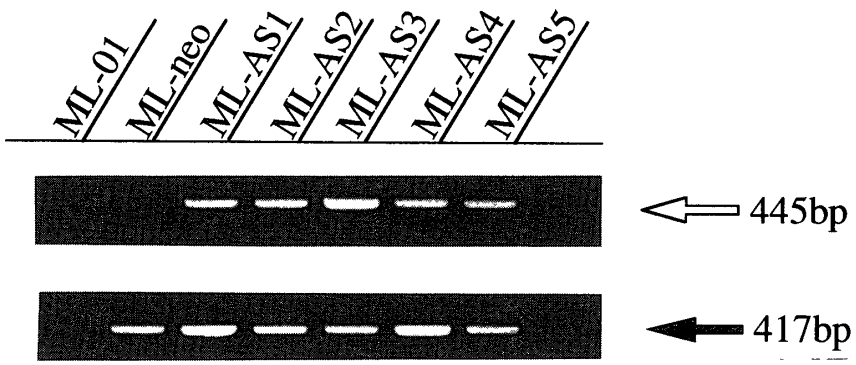

Fig. 2 PCR analysis of transfectants. Antisense Cu-Zn SOD cDNA was detected in all ML-AS clones (*Lane3-7*) as a band of 445bp (*open arrow*). Neomycin resitance gene DNA was detected in all ML-AS clones and ML-neo cells (*lane2-7*) as a band of 417bp (*closed arrow*).

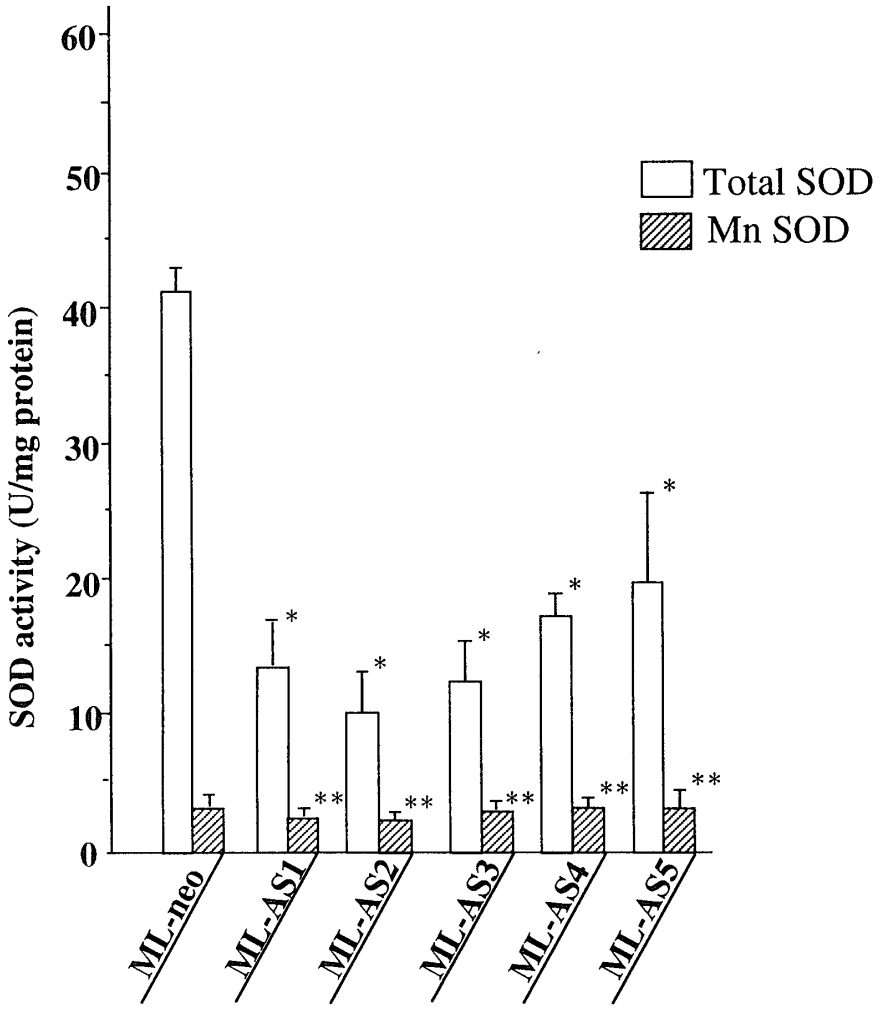

Fig. 3 Intracellular SOD activity of antisense ML-AS clones and ML-neo cells. The total SOD activity (□) was assayed by NBT method. Mn SOD activity (▨) was determined in the presence of 5mM sodium cyanide.
*, statistically significant ($p<0.01$) when compared to the ML-neo value.
**, statistically not significant when compared to the ML-neo value (Student's *t* test).

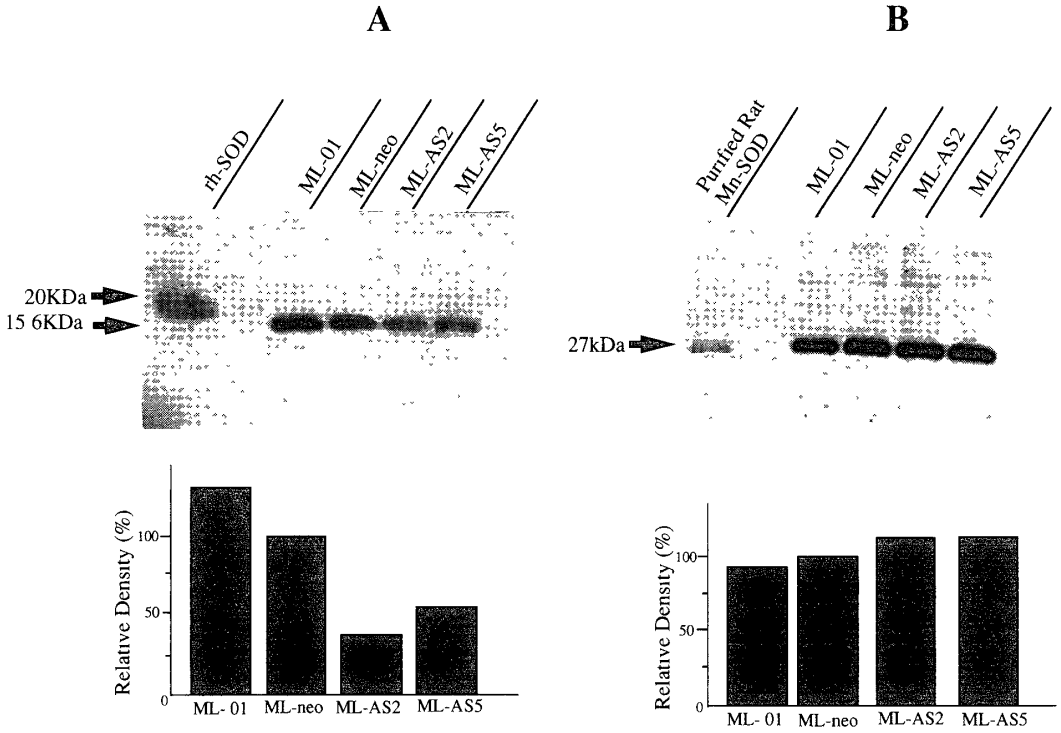

Fig. 4 SOD expression in ML-AS clones and ML-neo cells. Twenty μg of protein from whole cell extracts were subjected to SDS-PAGE, transferred to a nitorocellulose filter and reacted with anti-Rat Cu-Zn SOD antiserum (A) or anti-Rat Mn SOD antiserum (B)
A, The filter was reacted with anti-Rat Cu-Zn SOD antiserum The arrows indicates 20 KDarh-SOD and 15 6 KDa mouse Cu-Zn SOD.
B, The filter was reached with anti-Rat Mn SOD antiserum. The arrows indicates 27 KDa Rat liver Mn SOD
The bar graphs show the relative SOD expression level as determined by densitometric analysis, when the level of ML-neo was 100%.

parental cell, ML-01 and ML-Neo cells by immunoblotting using anti-rat Cu-Zn SOD antibody. When the blot was immunoreacted with anti-rat Cu-Zn SOD polyclonal antibody, densitometric assay revealed that the densities of 15.6kD bands in ML-AS2 and ML-AS5 were respectively 30% and 50% of that in ML-neo (Fig. 4A). Mn-SOD protein levels were also examined by immunoblotting and densitometric assay and there was no significant difference in the density of Mn-SOD band of each cell (Fig. 4B). These findings of immunoblotting were compatible with the above described results of SOD activity assay and clearly indicated that the intracellular Cu-Zn SOD activities of ML-AS clones were reduced by the transfection of the antisense Cu-Zn SOD cDNA.

Cell Motility of ML-AS Clones and ML-Neo Cells

Cell motility of ML-AS clones and ML-Neo cells were assayed using the Transwell® chamber. The proportion of cells which migrated through the filter was significantly higher in ML-AS5 and even higher in ML-AS2 than those in ML-01 and ML-neo. Motility of ML-AS2 and ML-AS5 were enhanced by superoxide generated by co-incubation of HPX and XOD, but

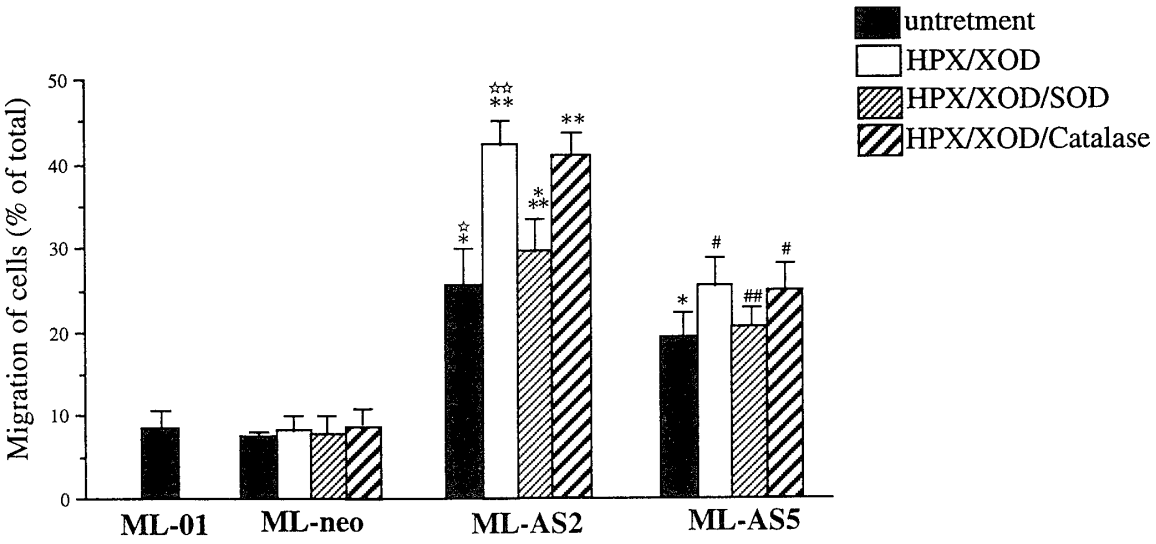

Fig. 5 Motility of ML-AS clones and ML-neo cells. Motility of cells was studied using Transwell® chamber as described in the method section. Percent migrated cells is shown for ML-neo cells, ML-AS2 clone and ML-AS5 clone of untreated group, superoxide generated by HPX and XOD treated group, superoxide plus rh Cu-Zn SOD (640U/ml) treated group and superoxide plus catalase (120U/ml) treated groups. Values are mean ±S. D. of triplicate samples from one representative experiment out of three experiments
*, $p<0.01$ statistically significant when compared to the ML-neo value.
**, $p<0.01$ statistically significant when compared to the untreated ML-AS2 value.
$\overset{*}{**}$, $p<0.01$ statistically significant when compared to ML-AS2 HPX/XOD value.
#, $p<0.05$ statistically significant when compared to the untreated ML-AS5 value
##, $p<0.05$ statistically significant when compared to ML-AS5 HPX/XOD value.
☆, $p<0.05$ statistically significant when compared to untreated ML-AS5 value.
☆☆, $p<0.01$ statistically significant when compared to ML-AS5 HPX/XOD value.
(Student's *t* test).

not that of ML-neo. The increment of motility was greater in ML-AS2 than in ML-AS5. The effect of superoxide was counteracted by rh Cu-Zn SOD added in the culture, but not by catalase, a scavenger of H_2O_2 (Fig. 5). These findings indicate that superoxide stimulates motility of Meth A cells with low intracellular Cu-Zn SOD activity but not those with high intracellular Cu-Zn SOD activity.

Cell Attachment of ML-AS clones and ML-neo cells

Cell attachment may be one of the factors which define the initial step of metastasis. In the present study, however the cell attachment to a Transwell® filter membrane made of polycarbonate revealed no difference between ML-AS clones and ML-neo cells (Fig. 6).

In Vivo and *in Vitro* Growth of Transfectants

In vitro proliferation assay revealed no significant difference in the doubling time of the antisense transfectants and parental cell or mock transfectant (Table 1). *In vivo* growth assay in syngeneic BALB/c mice also showed no significant difference among them (Fig. 7).

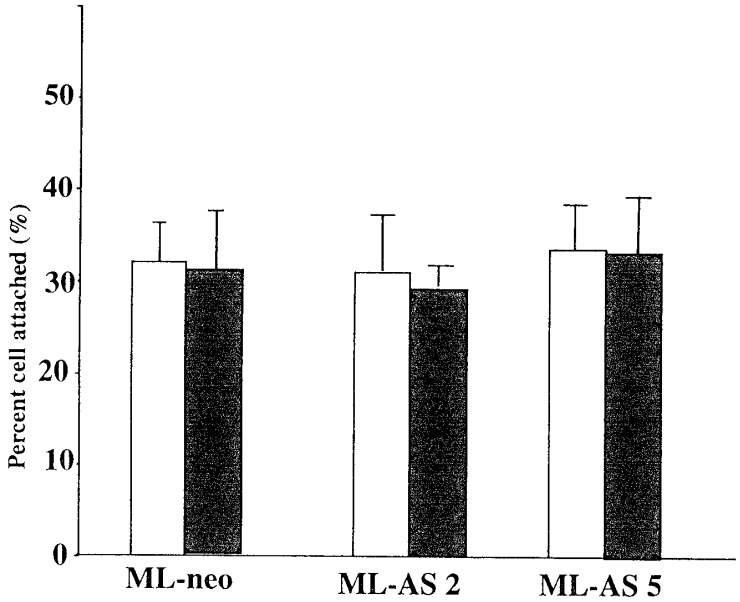

Fig. 6 Attachment of transfectants to the polycarbonate-made filter of Transwell® chamber. Attachment assay was performed using ^{125}I-IUDR labelled tumor cells under the condition with (■) or without (□) of HPX/XOD. Percent cell attachmed was calculated from the ratio of radioactivity of tumor cells which attached to the filter and total cells added in the upper chamber of a Transwell. There was no significant difference among the adhesivness ML-AS2, ML-AS5 and ML-neo

Table 1 *In vitro* proliferation of Meth A transfectants.

Cell line	Doubling time (h)
ML-01	23.6±1.4
ML-neo	22.3±1.5
ML-AS2	23.1±1.0
ML-AS5	24.3±2.1

Tumor cells were cultured as described in the method section. Number of living cells were counted at certain time intervals, under a microscope using trypan blue dye exclusion method.

Experimental Metastasis of ML-AS Clones and ML-Neo Cells

The effect of suppression of intracellular Cu-Zn SOD activity by antisense transfection on metastatic ability was examined. I performed an artificial metastasis assay. The mice injected with ML-AS2 or ML-AS5 showed significantly increased metastatic nodules (2.1 fold for ML-AS5 and 4.5 fold for ML-AS2) as compared to ML-neo cells (Table 2).

Discussion

It has been well known that tumor invasion and metastasis are defined by various factors of tumor cell and host. One of them, superoxide accelerates tumor invasion and metastasis by promoting cell motility. However the superoxide is eliminated by superoxide dismutase

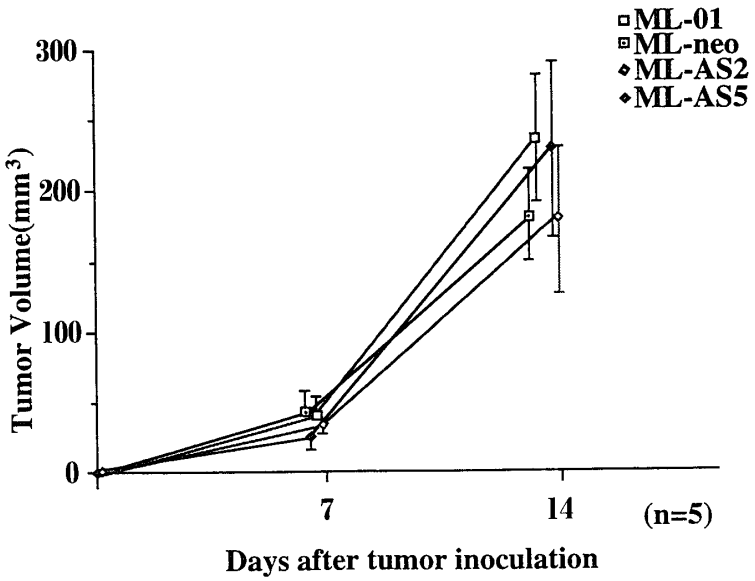

Fig. 7 Proliferation of Meth A antisense SOD transfectants in vivo. Cells (1.0×10^6) were inoculated into the right abodominal flank of BALB/c mice. Tumor volume was calculated by the formula $0.4 \times ab^2(mm^3)$, where a is the larger and b the smaller of the two dimensions. Values are mean ±S. D. (5mice/group).

Table 2 Experimental pulmonary metastasis of Meth A cells.

Cell line	Incidence of lung colonies	No.of lung colonies per mouse	Range
ML-01	10/10	40.8±18.9	19-71
ML-neo	10/10	41.2±18.0	10-63
ML-AS2	10/10	184.0±36.7 a. c	111-240
ML-AS5	10/10	86.8±22.2 b.	51-122

a. Statistically significant ($p<0.01$) when compared to ML-neo and ML-01.
b. Statistically significant ($p<0.05$) when compared to ML-neo and ML-01.
c. Statistically significant ($p<0.01$) when compared to ML-AS5.

(SOD). Utilizing antisense transfectants, I have confirmed that as Muramatsu *et al.*[8] previously observed with human oral cancer cells, that intracellular SOD (Cu-Zn SOD) regulates cell motility of murine fibrosarcoma as well. This confirmation on different cell types from different species led me to speculate that an inverse correlation between SOD activity and cellular motility may be a general biological phenomenon. In fact, my coleagues have recently observed that the motility of human macrophage is also enhanced by superoxide (unpublished observation).

Since motility is well acknowledged as one of the cellular factors which define metastatic potential[15,16], I intended to prove the direct linkage of intracellular SOD and metastasis in an artificial metastatic model of mouse. The result was that the transfectants in which Cu-Zn SOD activity was suppressed by antisense cDNA apparently exhibited significantly higher metastatic ability, 2.1 fold for ML-AS5 and 4.5 fold for ML-AS2, as compared to ML-neo cells.

In the present study, a spontaneous metastatic model was not employed, since a murine Meth A sarcoma never metastasized to the other site in host. Thus, to investigate the cell motility in vivo, an artificial metastatic model was considered good enough to provide sufficient information about invasion of tumor cells to the target organ (lung). And, I was able to demonstrate a sequential linkage of "SOD-motility-metastasis". With regard to SOD, which appeared to be independent from the superoxide scavenging activity, such as cell differentiation promoting activity[17] and suppression of malignant phenotype[18] have been recently reported. However, a motility-related SOD function is apparently attributable to the scavenging activity, since our antisense and the mock transfectant showed different reactivity to exogenously added superoxide. When an amount of superoxide in excess of what could be eliminated by the intracellular Cu-Zn SOD activity, was added from outside the cells (ML-AS2), the motility of the cell was accelerated. But when the cells (ML-neo) intrinsically contained a high level of Cu-Zn SOD activity, the motility of the cells remained low.

These facts, conversely, indicate that intracellular or extracellular superoxide itself is also a motility defining factor. Accordingly, my postulation that tumor cell motility (metastasis) would be accelerated in circumstances where superoxide is highly concentrated has been endorsed by my findings. This postulation may be verified also by previously reported findings that the inflammation[19], radiation treatment[20] or chemotherapy[21] which inevitably generate superoxide occasionally associated with an unexpected increment of metastasis. Further, the observation of Muramatsu *et al.*[8] that cytoplasmic Cu-Zn SOD, which is more readily accessible to extracellularly generated superoxide than Mn-SOD located predominantly in mitochondria, plays a more crucial role in cell motility than Mn-SOD, is also compatible with the above postulation.

Mechanism of enhancement of motility by superoxide is a matter of conjecture at present. Gene products such as NF-kB, which are induced by oxidant stress have been reported[22,23]. Those oxidant stress-inducible gene products may be coupled with an activation of cytoskeletal proteins (actin etc.). Modification or perturbation of celluar membrane may also be responsible for motility.

I admit further investigation needs to be done in order to elucidate the mechanism by which unscavenged superoxide enhance cell motility. The exploration of the intracellular Cu-Zn SOD level and *in vivo* metastatic potential of tumor cells may provide new insight for controlling metastasis by suppression of cell motility.

Conclusion

The present study was performed in order to comfirm whether or not antisense Cu-Zn SOD cDNA promotes motility and metastasis of murine Meth A sarcoma-derived ML-01 cells with low-metastatic property.

The results were as follows:

1. The intracelluar Cu-Zn SOD activity in the high metastatic MH-02 cells was significant less than that of the low metastic ML-01 cells.
2. The antisense transfected ML-AS clones exhibited decreased intracellular Cu-Zn SOD activity and increased motility as compared to ML-neo cells.
3. Superoxide generated by HPX and XOD enhanced the motility of ML-AS clones, but not of ML-neo cells.
4. When ML-AS clones were injected through the tail vein of mouse, the number of metastatic foci in lung showed significant increase as compared to ML-neo cells.
5. Above mentioned findings clearly proved that transfection of antisense Cu-Zn SOD cDNA promotes motility and metastasis of Meth A cells. And intracellular SOD are considered to be one of the factors defining tumor cell motility and metastatic ability.

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和 文 抄 録

以前，我々はヒト舌癌細胞において，高浸潤株は低浸潤株よりCu-ZnSOD活性が低く，さらにアンチセンスc-DNAを導入することによりCu-ZnSOD活性を抑制し，*in vitro*において細胞の運動能が亢進したことを報告した。しかしながら，この細胞内Cu-ZnSOD活性と腫瘍細胞の運動能との，逆相関関係は他の腫瘍細胞においても見られることなのか，また実際に*in vivo*での転移能は細胞内Cu-ZnSODにより規定されるのかは報告されていなかった。

今回，私はマウスMeth A細胞から樹立した低転移クローンML-01にCu-ZnSODのアンチセンスc-DNAを導入し5株のクローンを得た。そのうち最もSOD活性が低下したML-AS2，また活性の低下が最も少なかったML-AS5のSOD活性の異なる2つのクローンを用いて運動能と転移能を検討した。

結果，運動能についてはベクターのみを導入したML-neoと比較してML-AS2は4倍，ML-AS5は2.2倍の亢進が認められた。つぎに，ML-ASクローン群をSuperoxideにて処理したところ，運動能が促進したが，ML-neoでは変化を認めなかった。転移能はML-neoと比べるとML-AS2では4.5倍，ML-AS5では2.5倍の値を示した。

これらの結果から細胞内Cu-ZnSOD活性と転移能は，逆相関関係で細胞内Cu-ZnSODは運動能を規定する因子であることが示唆された。