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A novel clinical role for angiopoietin-1 in malignant pleural mesothelioma

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ABSTRACT: Malignant pleural mesothelioma (MPM) is an aggressive malignant tumour associated with asbestos exposure that has only a limited response to conventional therapy; therefore, diagnosing MPM early is very important. We have previously reported that angiopoietin (Ang)-1 was correlated with bleomycin-induced pulmonary fibrosis.

Here, we investigated the association of Ang-1 with the development of MPM cells, which originate from mesenchymal cells similar to lung fibroblasts, and demonstrated that Ang-1 stimulated the growth and migration of MPM cells *in vitro*. We also demonstrated that patients with MPM had significantly higher serum levels of Ang-1 in comparison to a population who had been exposed to asbestos but had not developed MPM. The patients with advanced-stage MPM showed higher levels of Ang-1 than the early-stage MPM patients and the Kaplan–Meier method revealed a significant correlation between serum Ang-1 levels and survival.

We propose the possibility that Ang-1 plays an important role in MPM tumour growth and our data suggest that the serum concentration of Ang-1 could be useful as prognostic factor.

KEYWORDS: Cytokines, pleural disease, serum marker

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Malignant pleural mesothelioma (MPM) is an aggressive, malignant tumour of mesothelial origin associated with asbestos exposure [1–3]. Although recently, asbestos usage has decreased in Western countries and Japan, the incidence of MPM is expected to increase markedly over the next few decades because there is a long latency period (20–40 yrs) between asbestos exposure and tumour development [4]. MPM shows only a limited response to conventional chemotherapy and radiotherapy. Although recently, the multitargeted antifolate pemetrexed has been approved as a first-line agent, in combination with cisplatin, for the treatment of MPM, overall survival remains very poor [5] with a median survival duration of 8–18 months [6]. In several centres, potentially curative surgery combined with some form of adjuvant therapy has been performed. Such early therapeutic intervention seems to be more beneficial than late intervention. Therefore, diagnosing MPM at an early stage is very important [1]. However, diagnosis can often be very difficult in histological studies. In the diagnosis of lung cancer, serum markers, such as carcinoembryonic antigen, cytokeratin fragment, progastrin-releasing peptide and squamous cell carcinoma antigen, provide supportive roles to confirm the diagnosis. Serum biomarkers for MPM, such as mesothelin and osteopontin [7, 8], have been reported and used to assist the diagnosis of MPM. For the further improvement of specificity and sensitivity of diagnosis, research into the development of novel biological markers is urgently required.

We have previously reported that angiopoietin (Ang)-1 was correlated with bleomycin-induced pulmonary fibrosis in mice [9]. In that report, we demonstrated that the Ang-1 mRNA level was increased in bleomycin-treated mouse lung tissues compared with that in control tissues. Moreover, we found that human lung fibroblasts and myofibroblasts produced Ang-1, which might be the underlying mechanism of pulmonary fibrosis. MPM involves the malignant transformation of mesothelial cells, which originate from mesenchymal cells similar to lung fibroblasts. Here, we investigated the effect of Ang-1 on the cell growth and/or migration of MPM cells *in vitro*, and the serum levels of Ang-1 in patients with MPM in comparison to a population that had been exposed to asbestos without developing MPM.

METHODS

Cell culture

The human MPM cell lines H28 (epithelioid), H2052 (sarcomatoid), H2452 (biphasic) and MSTO-211H (biphasic), and the human mesothelial cell line MeT-5A were obtained from the

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American Type Culture Collection (Rockville, MD, USA). These cells were cultured in RPMI 1640 (Sigma Chemical Co., St Louis, MO, USA) supplemented with 10% heat-inactivated fetal calf serum.

Quantitative real-time RT-PCR

Total RNA was isolated using the RNeasy Mini kit (QIAGEN, Valencia, CA, USA) and reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative, real-time RT-PCR was performed as previously described [10–12] using TaqMan Gene expression products for human Tie-2, Ang-1 and Ang-2. 18*S* ribosomal RNA served as an endogenous control (Applied Biosystems).

Measurement of Ang-1

Concentrations of Ang-1 and/or Ang-2 in cell culture supernatants and serum were measured using an ELISA kit (R&D Systems, Oxford, UK). The mean minimum detectable dose for Ang-1 of this ELISA kit is 3.45 $pg \cdot mL^{-1}$.

Measurement of total and phosphorylated Tie-2

Cells were cultured for 10 min with or without recombinant human Ang-1 (100 $ng \cdot mL^{-1}$) (R&D Systems), and cytoplasmic extracts were prepared using a nuclear extract kit (Active Motif, Carlsbad, CA, USA), then total and phosphorylated Tie-2 were detected using an ELISA kit (R&D Systems). We measured the total protein content in the cytoplasmic extracts (Protein Quantification Kit-Rapid, Dojindo, Kumamoto, Japan) and the values of total and phosphorylated Tie-2 were corrected as a proportion of the total protein content.

Cell proliferation assay

The cell proliferation assay was performed as previously described [9, 10, 12]. The MPM cell lines and MeT-5A cells were cultured in 96-well flat-bottomed culture plates for 2 days with or without recombinant human Ang-1 (1–100 $ng \cdot mL^{-1}$).

Cell migration assay

In vitro migration assays were performed using the CytoSelect 24-well Cell Migration Assay (8 μm, colorimetric format; Cell Biolabs, Huissen, The Netherlands), according to the manufacturer's instructions, as previously described [12]. Briefly, MPM cell lines and MeT-5A cells were suspended to a density of 1×10^6 $cells \cdot mL^{-1}$ in RPMI 1640 and placed in the upper half of a Boyden chamber. The lower half of the Boyden chamber was filled with RPMI 1640 containing 100 $ng \cdot mL^{-1}$ recombinant human Ang-1 or RPMI 1640 alone. The cells were incubated for 16 h, and nonmigratory cells that stayed in the upper chamber were removed. The migratory cells were stained with Cell Stain Solution, extracted using Extraction Solution, then measured using a plate reader at a wavelength of 560 nm.

Animals

6-week-old C.B-17/Icr-*scid* Jcl (*scid*/*scid*) severe combined immune deficiency (SCID) female mice were purchased from Clea Japan (Tokyo, Japan) and maintained in our specific pathogen-free animal facility. All animals were kept according to the Animal Protection Guidelines of Hyogo College of Medicine (Hyogo, Japan). All protocols for animal use and euthanasia were reviewed and approved by the Institute of Laboratory Animals (Graduate School of Medicine, Hyogo College of Medicine).

Ectopic (subcutaneous) xenograft model

To produce subcutaneous (*s.c.*) tumours, a single-cell suspension of 10^7 MSTO-211H cells was implanted *s.c.* into the back of SCID mice.

Immunohistochemistry

Mice were sacrificed 28 days after implantation. The tumours were fixed in 4% paraformaldehyde, dehydrated, embedded in paraffin and sectioned. Sections were immunostained by using rabbit anti-mouse Ang-1 polyclonal antibody (Fitzgerald, Concord, MA, USA) followed by anti- rabbit polyclonal antibody (Dako Cytomation, Glostrup, Denmark) and peroxidase activity was visualised using a diaminobenzidine kit (Dako Cytomation).

Patients

We studied the Ang-1 levels in the serum of 102 patients admitted for diagnosis to the Dept of Respiratory Medicine of Hyogo College of Medicine Hospital (Hyogo, Japan) from 2000 to 2007. All of the patients had a documented asbestos exposure history. 62 individuals had malignant pleural mesothelioma, which was diagnosed using histopathological samples by pathologists skilled in the diagnosis of MPM. 40 individuals had benign asbestos-related diseases (asbestosis or pleural plaques) or were healthy despite their asbestos exposure. All patients were classified by the staging system of the International Mesothelioma Interest Group (IMIG) [13]. Samples from 11 patients with lung cancer with malignant pleuritis and five healthy volunteers were also studied. The study was approved by our ethics committee in accordance with the 1975 Declaration of Helsinki. Informed consent was obtained from all patients. Samples were immediately frozen in liquid nitrogen and stored at -80°C until use.

Statistical analysis

For the *in vitro* study, data are presented as mean ± SD of three experiments performed in triplicate. Statistical analysis was performed using the Bonferroni–Dunn multiple comparisons test. The nonparametric Mann–Whitney U-test was used to compare two groups of serum samples. Comparisons of data between various groups of serum samples were performed with the nonparametric Kruskal–Wallis test followed by the Mann–Whitney U-test. In all tests, a p-value <0.05 was considered significant. In order to estimate the value of serum Ang-1, receiver operating characteristic (ROC) curves, areas under the ROC curves (AUC), and their 95% CI were calculated using standard techniques. To examine the cut-off values of serum levels, we calculated the total sensitivity and specificity for each cut-off value and then chose the cut-off values that maximised each factor. Estimates of the probability of survival were calculated using the Kaplan–Meier method and compared using the log-rank test. In order to evaluate the Ang-1 prognostic significance on survival of patients with MPM, Cox's proportional hazards regression analysis (backward) was carried out as multivariate analysis.

RESULTS

Evaluation of Ang-1, Ang-2 and Tie-2 expression

We evaluated mRNA expression of Ang-1, Ang-2, and Tie-2 by real-time RT-PCR analysis. As shown in table 1, Ang-1 mRNA was expressed in H2052 and MSTO-211H cells. However, H28, H2452 and MeT-5A showed no Ang-1 mRNA expression. Tie-2 mRNA was expressed in H2452, MSTO-211H and MeT-5A cells. No MPM or MeT-5A cells expressed Ang-2. Protein levels of Ang-1 and Ang-2 of the cells were analysed by ELISA. H2052 and MSTO-211H cells were demonstrated to produce Ang-1 (2,384.4±372.6 and 140.9±22.1 pg·mL^{-1}, respectively), while H28, H2452 and MeT-5A showed no Ang-1 production. By contrast, none of them produced Ang-2 (data not shown). We also examined total and phosphorylated Tie-2, and found that in MSTO-211H cells, phosphorylated Tie-2 was increased (1.2±0.036-fold increase, p=0.026), whereas total Tie-2 was not affected by the addition of Ang-1 (1.0±0.0028-fold increase). However, neither total nor phosphorylated Tie-2 was changed by the addition of Ang-1 in H2452 (1.0±0.052-fold increase and 1.0±0.016-fold increase, respectively) and MeT-5A (1.0±0.0060-fold increase, 1.1±0.052-fold increase; p=0.060, respectively) cells. Tie-2 was not detected in H28 and H2052 cells.

Effect of Ang-1 on the proliferation of MPM and MeT-5A cells

To clarify the involvement of Ang-1 in the development of MPM tumour growth, we studied the effect of Ang-1 on the proliferation of MPM cells and the human mesothelial cell line MeT-5A. As shown in figure 1, the addition of Ang-1 stimulated growth of MSTO-211H cells, which expressed both Ang-1 and Tie-2, and H2452 cells, which expressed only Tie-2, in a dose-dependent manner and reached a plateau at a concentration of 100 ng·mL^{-1} (1.3±0.045-fold (p<0.0001) and 1.1±0.014-fold increase (p<0.0001), respectively). The proliferation of H2052 cells, which expressed Ang-1 without Tie-2 expression, and H28 cells, which expressed neither, was not affected by the addition of Ang-1 (1.0±0.019-fold and 1.0±0.020-fold increase, respectively). However, Ang-1 had no effect on the proliferation of human mesothelial cell line MeT-5A, despite Tie-2 expression (1.0±0.049-fold increase, fig. 1).

Effect of Ang-1 on the migration of MPM cells

It is well known that cell migration plays an important role in tumour cell invasion, especially in the spread of MPM tumours. We therefore performed an *in vitro* migration assay to study the effect of Ang-1 on MPM progression and revealed that in both MSTO-211H, which express both Ang-1 and Tie-2, and H2452, which express only Tie-2, cell migration was induced (1.4-fold increase (p=0.002) and 1.3 fold increase (p=0.002), respectively) by Ang-1 (fig. 2). The migration of H2052 cells, which expressed Ang-1 but not Tie-2, and H28 cells, which expressed neither, was not induced by the addition of Ang-1 (data not shown). Ang-1 had no effect on the migration of MeT-5A mesothelial cells, despite expressing Tie-2 (fig. 2).

TABLE 1 Expression of angiopoietin (Ang)-1, Ang-2 and Tie-2 in malignant pleural mesthelioma and MeT-5A cells

mRNA	Cell line				
	H28	H2052	H2452	MSTO-211H	MeT-5A
Ang-1	-	+	-	+	-
Ang-2	-	-	-	-	-
Tie-2	-	-	+	+	+

+: expression; -: no expression.

Immunohistochemical findings

We previously reported that only MSTO-211H cells could grow *s.c.* in SCID mice when a single cell suspension of 10^7 H28, H2052, H2452, and MSTO-211H cells with a viability of >95% was implanted into the back of SCID mice [12]. To examine the pathophysiological roles of Ang-1 *in vivo*, tumour tissue from the ectopic xenograft model implanted with MSTO-211H cells was used for Ang-1 staining. Ang-1 staining could be seen in the cytoplasm of the tumour cells (fig. 2).

Serum levels of Ang-1 in patients with MPM, benign asbestos-related diseases (asbestosis or pleural plaques), and healthy individuals with a history of asbestos exposure

We recruited a total of 102 subjects with a history of asbestos exposure. Of them, 62 had confirmed MPM, 25 had pleural plaques and/or asbestosis, and 15 had no asbestos-related lesions despite their exposure to asbestos; *i.e.* they were healthy. Their characteristics are shown in table 2.

ROC curves for serum Ang-1 levels showed that patients with MPM had an AUC of 0.7974 (95% CI 0.7261–0.8687) in comparison to those with benign asbestos-related diseases (asbestosis or pleural plaques) and those who were healthy despite their asbestos-exposure. At a cut-off value of 34.5 ng·mL^{-1}, the diagnostic sensitivity was 71.0% and the specificity was 77.5% (fig. 3). The positive predictive value (PPV) was 83.0 %, and negative predictive value was 63.3%. The level of Ang-1 in patients with MPM was significantly higher (44.4±16.1 ng·mL^{-1}) in comparison to those with benign asbestos-related diseases (asbestosis or pleural plaques) and those who were healthy (28.5±10.7 ng·mL^{-1}; p<0.0001). Serum levels of Ang-1 from the patients with lung cancer with malignant pleuritis (n=11) were lower (31.9±9.1 ng·mL^{-1}; p=0.0088) than in MPM patients (fig. 3). Moreover, the scatter plots of the serum Ang-1 levels in MPM showed a tendency to increase as the disease progressed (stage I 35.7±4.7 ng·mL^{-1}, stage II 36.1±11.4 ng·mL^{-1}, stage III 40.2±13.7 ng·mL^{-1} and stage IV 49.3±17.2 ng·mL^{-1}; p=0.049 by the nonparametric Kruskal–Wallis test followed by the Mann–Whitney U-test; fig. 3). The differences in Ang-1 levels between the different MPM histological stages were not statistically significant (data not shown). There were no significant differences in Ang-1 levels among the subjects with benign asbestos-related diseases (asbestosis or pleural plaques) and those who were healthy despite having a history of asbestos-exposure (data not shown).

Relationship between Ang-1 levels and overall survival

To study the relationship between serum Ang-1 levels and the patients' clinical courses, we separated the patients based on

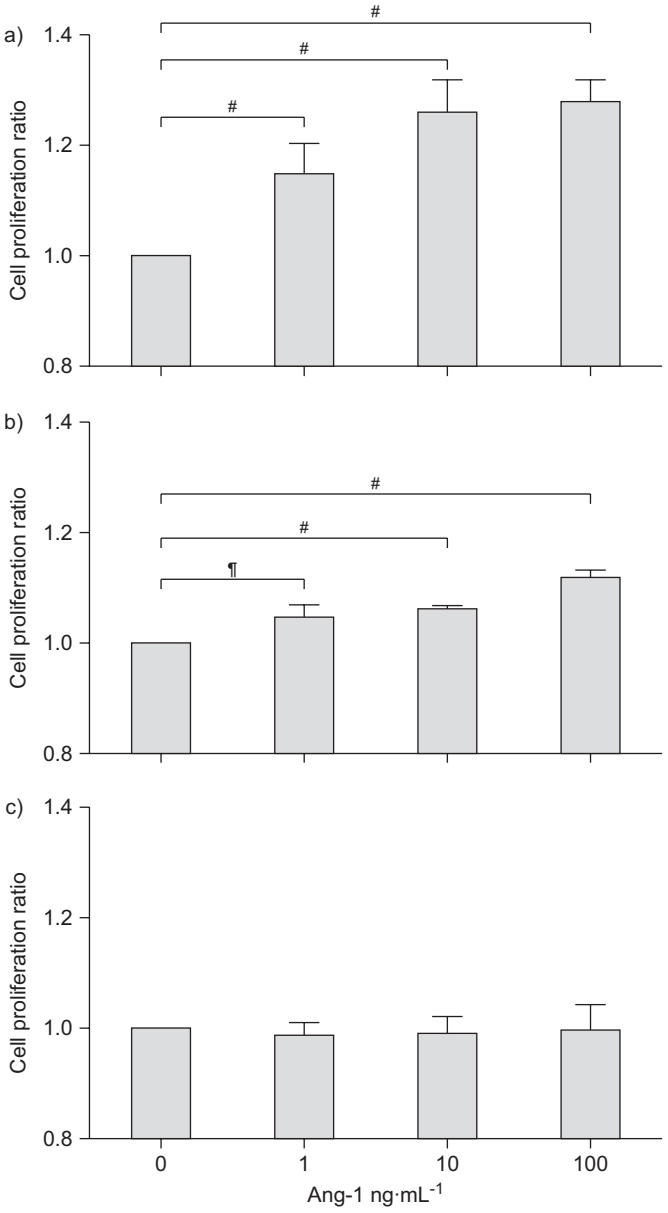

FIGURE 1. Effect of angiopoietin (Ang)-1 on the proliferation of malignant pleural mesothelioma (MPM) and mesothelial cells. a) MSTO-211H, b) H2452 MPM cells and c) the human mesothelial cell line MeT-5A were cultured in 96-well, flat-bottomed culture plates for 48 h in serum-free medium with 0, 1, 10 or 100 ng·mL^{-1} of Ang-1, and cell proliferation was assayed as described in the Methods section. Data are presented as mean±SD of three individual experiments performed in triplicate. Statistical analysis was performed using the Bonferroni–Dunn multiple comparisons test. #: $p<0.0001$. ¶: $p=0.0003$.

their serum Ang-1 levels at the time of the first measurement. The first group included patients with serum Ang-1 levels <34.5 ng·mL^{-1}, the cut-off value that we used. In this group of 13 patients, the mean serum Ang-1 value was 29.2±4.7 ng·mL^{-1}. The other group included the remaining 37 patients with serum Ang-1 levels ⩾34.5 ng·mL^{-1}, whose mean serum Ang-1 value was 52.5±14.2 ng·mL^{-1}. The difference in overall survival between the groups with lower and higher serum Ang-1 values than the assumed cut-off point of 34.5 ng·mL^{-1} was significant ($p=0.029$; fig. 4).

Cox's regression analysis was performed for 50 MPM patients for whom data on age, sex, histology, disease stage, performance status, treatments (pemetrexed-based chemotherapy or surgery) and serum Ang-1 level were available. An independent, statistically significant, prognostic effect on the survival of age (⩾65 *versus* <65 yrs of age; hazard ratio (HR) 2.63, 95% CI 1.04–6.68; $p=0.042$), pemetrexed-based chemotherapy (with *versus* without; HR 0.38, 95% CI 0.15–0.941; $p=0.035$) and serum Ang-1 level (⩾34.5 *versus* <34.5 ng·mL^{-1}; HR 3.43; 95% CI 1.08–10.87; $p=0.036$) was found.

DISCUSSION

MPM is a malignant transformation caused by the exposure of mesothelial cells to asbestos. It has a limited response to conventional chemotherapy and radiotherapy, and its prognosis is very poor. The lifetime risk of MPM is associated with occupational and/or environmental asbestos exposure history [14]. Due to the long latency period (typically >30 yrs) between first asbestos exposure and the onset of the disease, the diagnosis of MPM remains difficult, with incidence increasing all over the world [1, 2].

Although in advanced cases, resection of the tumour only prolongs survival by ~3 months, patients with stage IA [13] disease survive for ⩾5 yrs after total resection of the tumour [8]. We have screened outpatients with a history of asbestos exposure but no symptoms for several years in order to detect MPM in its early stages. Due to the difficulty of the early diagnosis of MPM by radiological and/or histological examinations, efficient and practical serum biomarkers are required to aid the diagnosis of MPM. To date, there have been several reports on candidates for clinically useful markers for MPM [7, 8, 15–17]. Indeed, some of them have been reported to be useful serum markers of MPM, such as mesothelin and osteopontin [7, 8, 15]; however, little is known about their biological functions or effects on MPM cells.

Ang-1 and -2 are counteracting ligands for the endothelial-specific receptor tyrosine kinase Tie-2, and are important regulators of blood vessel growth, maturation and function. Ang-1 promotes angiogenesis, induces vascular maturation, and decreases vascular permeability. However, Ang-2 has the ability to destabilise blood vessels and enhance vascular leaking, and it antagonises Ang-1 [18–20].

We previously reported that Ang-1 is associated with bleomycin-induced pulmonary fibrosis in mice [9]. MPM is a malignant transformation of mesothelial cells, which originate from mesenchymal cells similar to lung fibroblasts; therefore, we investigated the effects of Ang-1 on mesothelial cells.

First, we demonstrated the production of Ang-1 in two out of four MPM cell lines, and expression of mRNA of its receptor, Tie-2, in two of them. The human mesothelial cell line Met-5A, which was used as a control, expressed Tie-2 mRNA, but not Ang-1. MSTO-211H cells expressed mRNA of both Ang-1 and its receptor. To clarify the involvement of Ang-1 in the development of MPM tumour growth, we studied the effect of Ang-1 on the proliferation of MPM cells and demonstrated that the addition of Ang-1-stimulated MPM cell growth in

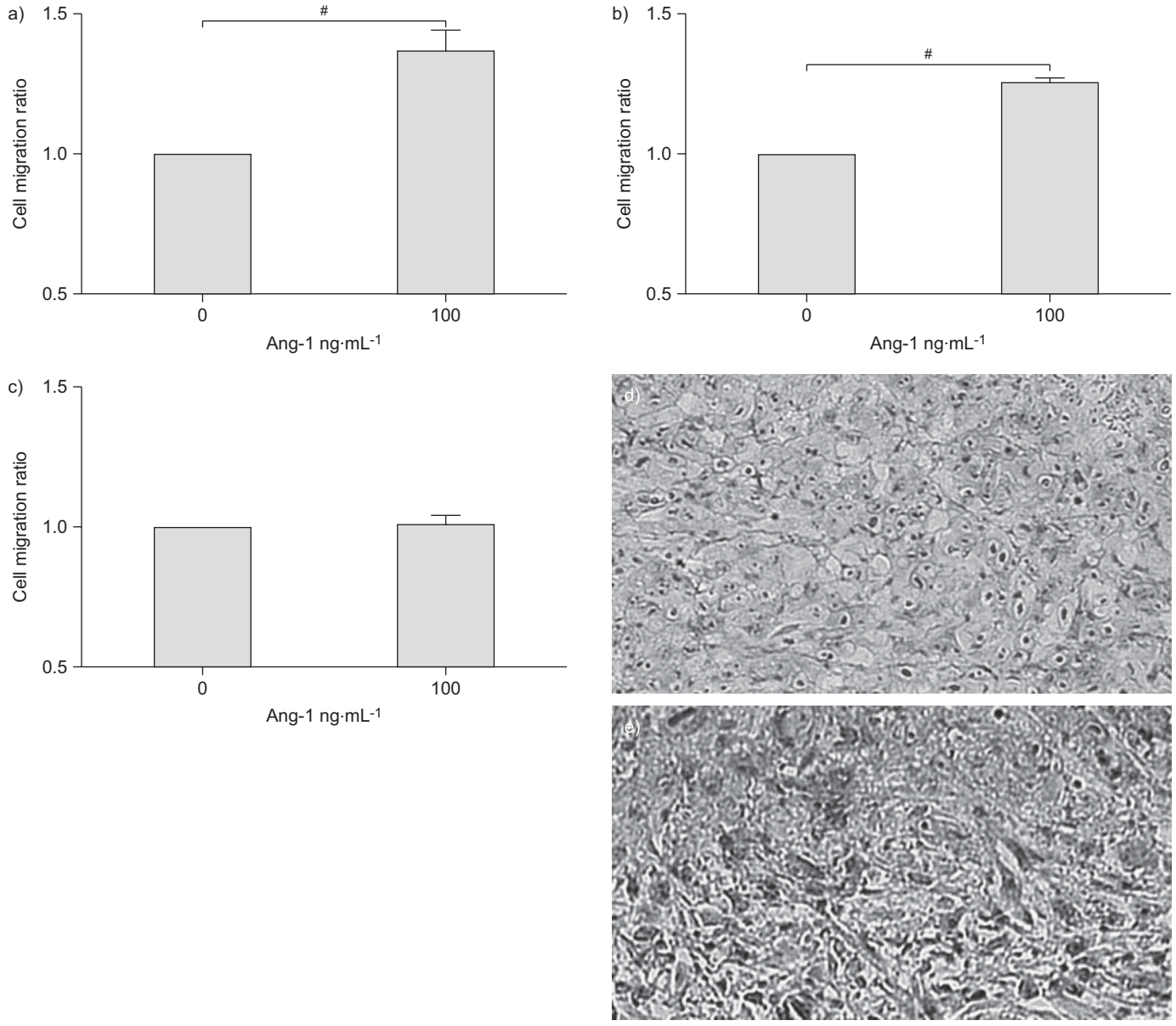

FIGURE 2. Effect of angiopoietin (Ang)-1 on the migration of malignant pleural mesothelioma (MPM) and mesothelial cells. a) MSTO-211H, b) H2452 MPM cells and c) the human mesothelial cell line MeT-5A were cultured overnight in the presence or absence of 100 ng·mL^{-1} Ang-1, and a cell migration assay was performed as described in Materials and Methods. Data are presented as mean±SD of three individual experiments performed in triplicate. Statistical analysis was performed using the Bonferroni–Dunn multiple comparisons test. The tumours from severe combined immune deficiency mice injected subcutaneously with MSTO-211H cells were fixed in 4% paraformaldehyde, dehydrated, embedded in paraffin and sectioned. Sections were immunostained using d) control or e) rabbit anti-mouse Ang-1 polyclonal antibody as described in the Methods section. #: p=0.002. Original magnification ×400.

MSTO-211H and H2452 cells, which expressed Tie-2, in a dose-dependent manner. However, Ang-1 had no effect on the proliferation of MPM cell lines H2052 and H28, which lacked Tie-2 expression, suggesting the functional importance of Tie-2 in Ang-1-induced proliferation. Ang-1 also had no effect on the proliferation of MeT-5A, suggesting the absence of functional expression of Tie-2 in normal mesothelial cells, although it might become functional when the cells are transformed. The present data suggest the proliferation of MPM cells is increased *via* the Ang-1 autocrine and/or paracrine mechanisms of MPM cells and Ang-1-producing surrounding cells, such as fibroblasts [9] and pericytes [19].

Cell migration plays an important role in tumour cell invasion and we previously demonstrated the important role of MPM cell migration in tumour growth [9]. We therefore performed an *in vitro* migration assay to study the effect of Ang-1 on MPM spreading and revealed that in MSTO-211H and H2452 cells, cell migration was induced by Ang-1. The migration of H2052 or H28 cells, which lacked Tie-2 expression, was not induced by the addition of Ang-1, suggesting the importance of Tie-2 also in Ang-1-induced migration. However, Ang-1 had no effect on the migration of MeT-5A mesothelial cells, suggesting a limited effect of Ang-1 on MPM cells. Based on these findings, one possible mechanism is that MPM cell spread is

TABLE 2 Characteristics of malignant pleural mesothelioma (MPM) patients and non-MPM subjects with asbestos exposure

MPM#	
Cases	62
Age yrs	65.45±9.18
Males/females	46/16
Histology	
Epithelioid	44
Sarcomatoid	8
Biphasic	5
Desmoplastic	5
Stage I/II/III/IV	6/7/13/36
Non-MPM#	
Cases	40
Age yrs	69.05±8.65
Males/females	33/7
CT findings	
Plaque	20
Asbestosis	3
Plaque and asbestosis	2
None	15

Data are presented as n or mean±SD. CT: computed tomography. #: all of the individuals were exposed to asbestos.

triggered by the Ang-1 produced by the MPM cells themselves and/or Ang-1-producing surrounding cells.

Moreover, it is well known that malignant tumours require new blood vessel formation, and it has been reported that increased vascularity in MPM is associated with a poor prognosis [2]. Ang-1 is also an important regulator of blood vessel growth, maturation and function. Ang-1 promotes angiogenesis, induces vascular maturation and decreases vascular permeability [18–20].

Although the precise cellular mechanism has not been fully investigated, we propose the possibility that Ang-1 plays an important role in MPM tumour growth through stimulation of both proliferation and cell migration. Of course, as the main function of Ang-1 is the promotion of angiogenesis, we consider that in addition to its effects on the proliferation and migration of MPM cells, increased angiogenesis might be one of the mechanisms involved in Ang-1-induced growth of MPM tumours [21].

Next, we evaluated the clinical role of Ang-1 as a serum biomarker for MPM. In this study, we found that patients with MPM had significantly higher serum levels of Ang-1 in comparison to a population with a history of asbestos exposure, or healthy volunteers (n=5; age 32.0±8.0 yrs; three males and two females) never exposed to asbestos (Ang-1 33.7±4.3 ng·mL^{-1}). Serum Ang-1 levels between a population with a history of asbestos exposure and healthy volunteers never exposed to asbestos were not statistically significant. Although the diagnostic sensitivity of Ang-1 for MPM according to the ROC curve was not high (71.0%), PPV was

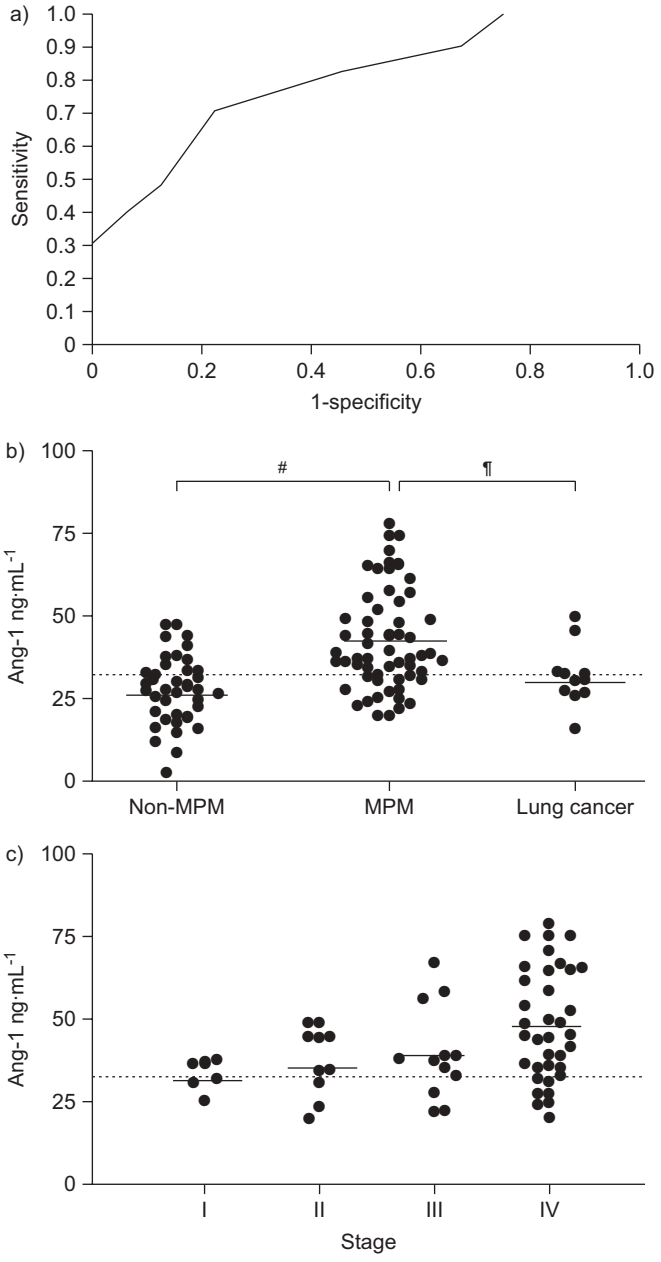

FIGURE 3. Serum angiopoietin (Ang)-1 levels in patients with malignant pleural mesothelioma (MPM) and non-MPM subjects. a) Sensitivity and specificity of serum Ang-1 for distinguishing patients with MPM from non-MPM subjects (receiver operating characteristic curve). An analysis that included 62 MPM patients and 40 non-MPM subjects with a history of asbestos exposure gave an area under the curve of 0.7974 (95% CI 0.7261–0.8687). At a cut-off value of 34.5 ng·mL^{-1}, the diagnostic sensitivity was 71.0% and the specificity was 77.5%. b) Serum levels of Ang-1 in non-MPM subjects, MPM patients and lung cancer with malignant pleuritis were measured as described in the Methods section. c) Serum levels of Ang-1 in MPM patients divided into four disease stages are shown. The b) nonparametric Mann–Whitney U-test or the c) nonparametric Kruskal–Wallis test followed by the Mann–Whitney U-test was used. ——: mean value of each group. ·····: cut-off value. #: $p<0.0001$. ¶: $p=0.0088$.

fairly good (83.0 %), suggesting that the high serum Ang-1 levels might be supportive for the diagnosis of MPM. We also measured serum levels of Ang-1 from the patients with lung

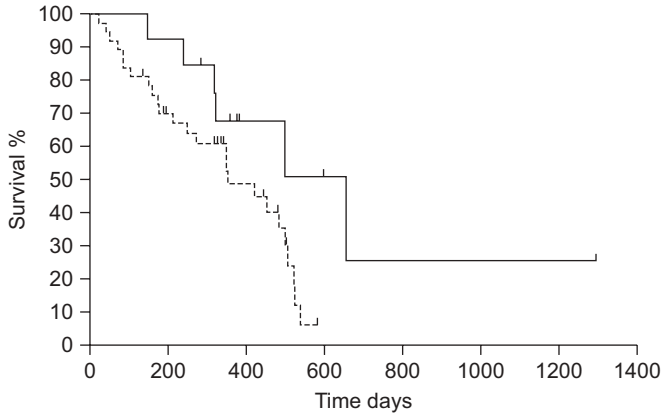

FIGURE 4. Survival of malignant pleural mesothelioma subjects according to serum angiopoietin (Ang)-1 levels. Estimates of the probability of survival were calculated using the Kaplan–Meier method and compared using the log-rank test. ——: 34.5 ng·mL^{-1}; ------: ⩾34.5 ng·mL^{-1}.

cancer with malignant pleuritis and found lower Ang-1 levels than MPM patients.

Moreover, the levels gradually increased with disease progression, although only stage IV MPM had potentially significant higher values of Ang-1, and the Kaplan–Meier method revealed a significant correlation between serum Ang-1 levels and survival. Furthermore, serum Ang-1 level was revealed to have a statistically significant, prognostic effect on survival by Cox's regression analysis, which suggested its usefulness as a marker to estimate prognosis. Since the clinical stage of MPM is not related to the presence or absence of pleural effusion, and the early distinction of MPM patients from those with benign asbestos-related diseases is necessary, we propose that measuring the Ang-1 levels in serum seems an easy and useful tool for clinical management for MPM.

In summary, we demonstrated that Ang-1-stimulated the cell growth and migration of MPM cells in *in vitro* studies. We also demonstrated that patients with MPM had significantly higher serum levels of Ang-1 in comparison to a population with a history of asbestos exposure that did not develop MPM, and the patients with advanced-stage MPM showed higher levels of Ang-1 compared to patients with early stage MPM. We suggest that Ang-1 could be a novel useful serum prognostic factor. This is the first report on the relationship between Ang-1 and MPM.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

None declared.

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