Respiratory Medicine (2011) **105**, 137–142

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

Pleural effusion VEGF levels as a prognostic factor of malignant pleural mesothelioma

Noriko Hirayama [a,c], Chiharu Tabata [a,*,c], Rie Tabata [b], Risa Maeda [a], Akihiro Yasumitsu [a], Shusai Yamada [a], Kozo Kuribayashi [a], Kazuya Fukuoka [a], Takashi Nakano [a]

[a] *Division of Respiratory Medicine, Department of Internal Medicine, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663 8501, Japan*
[b] *Department of Internal Medicine, Hyogo Prefectural Tsukaguchi Hospital, Hyogo, Japan*

Received 3 June 2010; accepted 7 October 2010
Available online 30 October 2010

KEYWORDS
Mesothelioma;
Tumor marker;
Cytokine

Summary
Background: Malignant pleural mesothelioma (MPM) is an aggressive malignant tumor of mesothelial origin associated with asbestos exposure. MPM has a limited response to conventional chemotherapy and radiotherapy so early diagnosis of MPM is very important. Vascular endothelial growth factor (VEGF), a potent mitogen for the vascular endothelium, is also known to be an autocrine growth factor for MPM. Here, we investigated the pleural effusion VEGF levels in patients with MPM and compared them to those of a population with a non-malignant pleuritis or lung cancer involving malignant pleural effusion.
Methods: The pleural effusion VEGF concentrations were measured in 46 MPM patients and 45 individuals with non-MPM individuals (25 individuals with non-malignant pleural effusions, and 20 individuals with lung cancer involving malignant pleural effusion).
Results: We demonstrated that patients with MPM had significantly higher pleural effusion VEGF levels than a population with non-malignant pleuritis or lung cancer involving malignant pleural effusion, and the patients with advanced stage MPM showed higher levels of VEGF than the early stage MPM patients. The difference in overall survival between the groups with pleural effusion VEGF levels lower and higher than the assumed cut-off of 2000 pg/ml was significant.
Conclusions: Our data suggest that the pleural effusion VEGF concentration could be useful as an aid for the diagnosis of MPM and as a prognostic factor.

Abbreviations: AUC, area under the ROC curve; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; MPM, malignant pleural mesothelioma; PDGF, platelet derived growth factor; ROC, receiver operating characteristic; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

* Corresponding author. Tel.: +81 798 45 6596; fax: +81 798 45 6597.
E-mail address: ctabata@hyo-med.ac.jp (C. Tabata).
[c] These two authors contributed equally to this study.

0954-6111/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2010.10.010

Introduction

Malignant pleural mesothelioma (MPM) is an aggressive malignant tumor of mesothelial origin associated with asbestos exposure.[1–3] Although asbestos usage has recently decreased in Western countries and Japan, the incidence of MPM is expected to markedly increase over the next few decades because there is the long latency period (20–40 years) between asbestos exposure and tumor development.[4] MPM shows limited response to conventional chemotherapy and radiotherapy. Although the multi-targeted anti-folate pemetrexed has recently 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 centers, 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 early is very important.[1] However, cytological diagnosis of pleural effusions can often be very difficult in MPM because MPM cells can not be easily distinguished from lung cancer cells and sometimes they can not be detected in pleural effusions.[1] Pleural effusion biomarkers for MPM such as hyaluronic acid and CYFRA 21-1 have been reported and used to assist the diagnosis of MPM.[7, 8] To further improve the specificity and sensitivity of diagnosis, research into the development of novel biological markers is urgently required.

Tumor growth and metastasis are associated with angiogenesis. We previously reported that vascular endothelial growth factor (VEGF), a potent mitogen for the vascular endothelium, 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. Moreover, we recently reported that patients with MPM had significantly higher serum levels of VEGF than a population with a history of asbestos exposure without developing MPM, which suggested its usefulness as a marker for MPM.[10] In this study, we evaluated the clinical role of pleural effusion VEGF levels in MPM and found that patients with MPM had significantly higher pleural effusion VEGF levels than a population with a non-malignant pleuritis or lung cancer involving malignant pleural effusion, which suggested its usefulness as a marker for MPM. Our results are consistent with previous reports demonstrating that VEGF is expressed in MPM, and moreover, that it acts as an autocrine growth factor for MPM.[11]

Materials and methods

Patients and pleural effusion samples

We studied the VEGF levels in pleural effusions collected from 91 individuals presenting at the Department of Respiratory Medicine of Hyogo College of Medicine Hospital from 2005 to 2009. The pleural effusions were obtained by thoracocentesis. All patients were diagnosed by pathologists, and it was confirmed that their clinical course matched their diagnosis. Forty-six individuals had malignant pleural mesothelioma involving a documented asbestos exposure history. These cases were diagnosed by pathologists skilled in the diagnosis of MPM using histopathological samples. All patients were classified using the staging system of the International Mesothelioma Interest Group (IMIG).[12] Patients with MPM were treated according to our therapeutic guideline: combination chemotherapy including multi-targeted anti-folate pemetrexed was performed for patients with PS 0–1 and age < 70, and for the remainders, best supportive care was chosen. Surgical treatment was not performed for the patients in the present study. Twenty-five individuals had non-malignant pleural effusions, and 20 individuals had lung cancer involving malignant pleural effusion. The study was approved by our ethics committee in accordance with the 1975 Declaration of Helsinki. Informed consent was obtained from all patients. Fresh pleural effusion samples were collected before treatment and centrifuged for 10 min at 2000 g, before the resultant supernatants were immediately frozen in liquid nitrogen and stored at -80° until use.

Measurement of VEGF

The VEGF concentrations of the pleural effusions were measured using an enzyme-linked immunosorbent assay (ELISA) Kit (R&D Systems, Oxford, UK) according to the manufacturers' instructions.

Statistical analysis

The nonparametric Mann–Whitney *U*-test was used to compare two groups of samples. Comparisons of data between various groups 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 significance of the pleural effusion VEGF values, receiver operating characteristic (ROC) curves, the area under the ROC curve (AUC), and their 95% confidence intervals (95% CI) were calculated using standard techniques. To examine the cut-off values for the pleural effusion VEGF levels, we calculated the total sensitivity and specificity for each cut-off value and then chose the cut-off values that maximized each factor. Correlations between data were analyzed using Spearman's rank correlation test. Estimates of the probability of survival were calculated by the Kaplan–Meier method and compared using the log-rank test. In order to evaluate the prognostic significance of VEGF on the survival of patients with MPM, Cox's proportional hazards regression analysis was carried out as a multivariate analysis.

Results

VEGF pleural effusion levels in patients with MPM and individuals with non-MPM

We recruited a total of 91 subjects suffering from pleural effusion. Of them, 46 had confirmed MPM, 25 had no malignant pleural effusion, and 20 had lung cancer involving malignant pleural effusion. Their characteristics are shown in Table 1.

The ROC curves for the pleural effusion VEGF levels showed that the patients with MPM had an AUC of 0.8304 in

comparison to those with non-malignant pleural effusion (95% CI: 0.7528—0.9081). At the optimal cut-off value of 2000 pg/ml, the diagnostic sensitivity was 71.7%, and the specificity was 76.0% (Fig. 1A). The mean pleural effusion VEGF concentration of the patients with MPM was significantly higher (5303.6 ± 1711.0 pg/ml) than that of the patients with non-malignant pleural effusion and the patients with lung cancer involving malignant pleural effusion (1172.2 ± 1212.8 pg/ml, 2429.9 ± 2173.8 pg/ml) ($p = 0.0003$, $p = 0.0017$, respectively) (Fig. 1B). Moreover, scatter plots of the pleural effusion VEGF levels of the MPM patients showed statistically significant tendencies to increase as the stage increased (stage I: 1919.2 ± 1802.8 pg/ml, stage II: 3764.0 ± 2432.0 pg/ml, stage III: 3956.7 ± 2316.5 pg/ml, and stage IV: 4789.8 ± 2281.3 pg/ml) ($p = 0.025$, Fig. 1C). There were no statistically significant differences between the pleural effusion VEGF levels of the MPM histological groups (epithelioid: 4000.0 ± 2499.3 pg/ml, non-epithelioid: 4185.3 ± 2245.5 pg/ml) or sex (male: 4253.9 ± 2381.9 pg/ml, female: 3070.6 ± 2470.9 pg/ml), and there were no significant differences in the pleural effusion VEGF levels between the subjects with benign asbestos pleurisy and those with benign pleurisy without a history of asbestos exposure (1853.8 ± 1438.7 pg/ml, 1139.6 ± 1223.5 pg/ml, respectively).

Correlation of VEGF levels between pleural effusions and serum

We examined pleural effusion and serum VEGF levels in 16 MPM patients and showed that there was a significant correlation between them ($r = 0.51$, $p = 0.046$, Fig. 2). The numbers of MPM patients with higher VEGF levels in both their serum and pleural effusion; higher VEGF levels in their serum alone; higher VEGF levels in their pleural effusion alone; and levels lower than 460 pg/ml (serum cut-off level) and 2000 pg/ml (cut-off level for pleural effusion) in the serum and pleural effusion, respectively,[10] were 7, 5, 2, and 2, respectively. The two MPM patients with lower VEGF levels in both their serum and pleural effusion were classified as stage I.

Relationship between pleural effusion VEGF levels and overall survival

Among the 46 MPM patients, we were able to follow 28 patients closely for up to 600 days. To study the relationship between the pleural effusion VEGF levels and the patients' clinical courses, we separated the patients according to their pleural effusion VEGF levels at the time of the first measurement. The first group included patients with pleural effusion VEGF levels of lower than 2000 pg/ml (the cut-off value that we chose). In this group of 11 patients, the mean VEGF value was 1070.2 pg/ml (interquartile range: 634.3—1498.4). The other group included the remaining 17 patients with pleural effusion VEGF levels of higher than 2000 pg/ml, whose mean VEGF value of pleural effusions was 5101.1 pg/ml (interquartile range: 3184.9—6311.4). The difference in overall survival between the groups with lower and higher pleural effusion VEGF values than the assumed cut-off point of 2000 pg/ml was significant ($p = 0.041$, Fig. 3). Cox's regression analysis was performed on 28 MPM patients for whom data on age, sex, histology, stage, and the pleural effusion VEGF level were available. Independent

Table 1 Characteristics of the MPM patients and non-malignant subjects.

		Cases (%)	Total
MPM			
Age	69.17 ± 9.64		
Sex	Male/Female	38(82.6)/8(17.4)	46
Histology	Epithelioid	34(73.9)	
	Sarcomatoid	10(21.7)	
	Biphasic	2(4.4)	
Stage	I/II/III/IV	8(17.5)/6(13.0)/6(13.0)/26(56.5)	
No-malignancy			
Age	70.50 ± 13.02		
Sex	Male/Female	21(84.0)/4(16.0)	25
	Benign asbestos pleurisy	5(20.0)	
	Tuberculous(Tb) pleurisy	8(32.0)	
	Infectious (non-Tb) pleurisy	8(32.0)	
	Para-pneumonic	1(4.0)	
	Heart failure	1(4.0)	
	Hepatic failure	1(4.0)	
	Renal failure	1(4.0)	
Lung cancer			
Age	69.00 ± 8.84		
Sex	Male/Female	10(50.0)/10(50.0)	20
	Adenocarcinoma	17(85.0)	
	Squamous cell	2(10.0)	
	Small	1(5.0)	

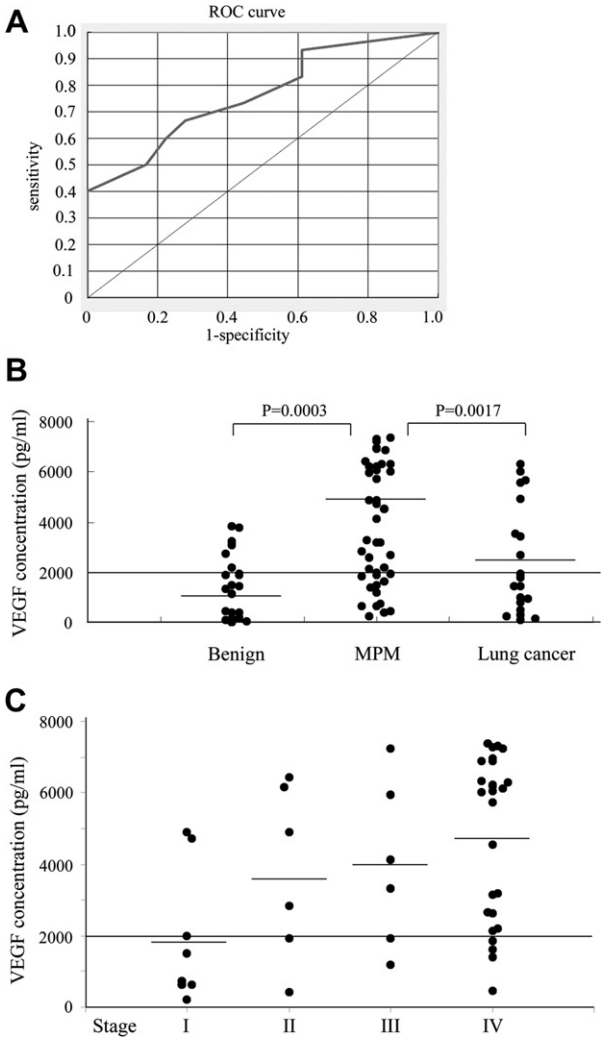

Figure 1 Pleural effusion VEGF levels in patients with MPM and non-MPM subjects. (A) An analysis that included 46 MPM and 25 non-malignant pleural effusion patients revealed an area under the curve (AUC) of 0.8304 (95%CI: 0.7528—0.9081). At the optimal cut-off value of 2000 pg/ml, the diagnostic sensitivity was 71.7%, and the specificity was 76.0%. (B) The pleural effusion VEGF levels of the patients with MPM versus those of the patients with non-malignant pleural effusion or lung cancer involving malignant pleural effusion were measured as described in Materials and Methods. (C) The pleural effusion VEGF levels in the MPM patients divided into 4 stages are shown. The nonparametric Mann—Whitney *U*-test (B) or the nonparametric Kruskal—Wallis test followed by the Mann—Whitney *U*-test (C) was used. *p*-values <0.05 were considered significant. The horizontal bars represent the mean of each group. The cut-off value is shown as a horizontal line.

statistically significant prognostic effects on survival were found for age (65 $\geq$ versus $<$ 65years; HR, 15.6; 95% CI, 1.068—229.125; $p = 0.045$), sex (male versus female; HR, 0.380.0031; 95% CI, 0.000062—0.1555; $p = 0.0038$), histology (sarcomatoid versus epithelioid; HR, 8.663; 95% CI, 1.114—67.351; $p = 0.0039$), and pleural effusion VEGF level (2000 $\geq$ versus $<$ 2000 pg/ml; HR, 961.23; 95% CI, 7.083—130446.4; $p = 0.0061$).

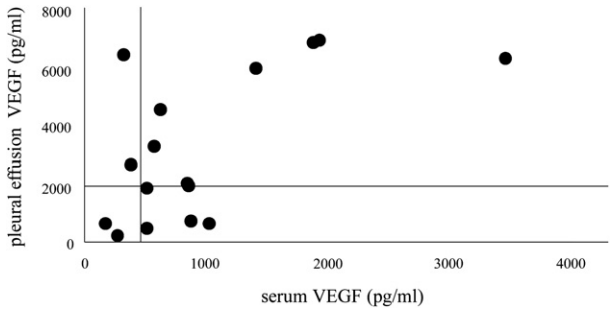

Figure 2 Correlation of VEGF levels between pleural effusions and sera. We examined the pleural effusion and serum VEGF levels in 16 MPM patients and showed that there was a significant correlation between the two ($r = 0.51$, $p = 0.046$). Correlations between data were analyzed using Spearman's rank correlation test.

Discussion

MPM is a malignant transformation caused by the exposure of mesothelial cells to asbestos, which shows 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.[13] Due to the long latency period (typically longer than 30 years) between the first asbestos exposure and the onset of the disease, MPM remains a universally fatal disease of increasing incidence all over the world.[1, 2]

Although in advanced cases, resection of the tumor only prolongs survival by about 3 months, patients with stage IA disease survive for five or more years after total resection of the tumor.[14] Due to the difficulty of the differential diagnosis of MPM among individuals with pleural effusion by radiological, cytological, and/or histological examinations, efficient and practical pleural effusion biomarkers are required to aid the diagnosis of MPM. To date, there have been several reports concerning candidates for clinically useful markers for MPM.[14—17] Indeed, the level of mesothelin-related protein (SMRP), the soluble form of mesothelin, has been reported to be a useful pleural effusion marker in MPM.[18] Mesothelin is thought to play a role in cell-adhesion, cell-to-cell recognition, and signaling; however, its biological functions in MPM cells have not been

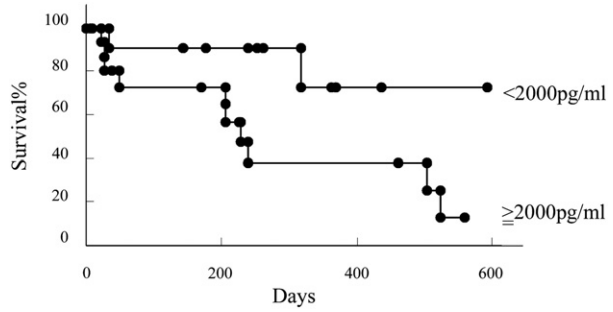

Figure 3 Survival of MPM subjects according to pleural effusions VEGF levels. Estimates of the probability of survival were calculated using the Kaplan—Meier method and compared using the log-rank test.

fully investigated and there was no significant difference between pleural effusion mesothelin levels and survival in MPM patients.[18]

Therefore, it is important to find biological markers with effects on MPM cells that are closely related to MPM progression. MPM cells are known to exhibit increased and/or dysregulated growth. Several factors, including transforming growth factor (TGF)-β_1, platelet derived growth factor (PDGF), TGF-α, and interleukin (IL)-8, have been reported to be associated with MPM cells.[19–24] Moreover, tumor development is closely related to angiogenesis. Malignant tumors require new blood vessel formation, and it has been reported that increased vascularity in MPM is associated with a poor prognosis.[2, 25] VEGF is known to be an important regulator of angiogenesis and plays critical roles in endothelial cell proliferation, vascular permeability, and angiogenesis in several inflammatory lesions.[26–28] We previously reported that VEGF 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, so it would not be surprising if VEGF was found to be associated with MPM progression. Moreover, we recently reported that patients with MPM had significantly higher serum levels of VEGF than a population with a history of asbestos exposure, which suggested its usefulness as a marker for MPM. In this study, we evaluated the clinical role of VEGF as a pleural effusion biomarker in MPM and found that patients with MPM had significantly higher pleural effusion VEGF levels than a population with non-malignant pleural effusion or lung cancer involving malignant pleural effusion.

Paracrine and autocrine mechanisms have been reported for several cytokines in malignant tumors. We recently reported the autocrine and/or paracrine mechanism of TGF-β_1 in MPM.[19] VEGF has also been reported to be an autocrine growth factor of MPM.[11, 29] On the other hand, Li et al.[30] reported that treatment with anti-VEGF neutralizing antibody suppressed MPM progression in a mouse model, mainly by preventing angiogenesis, especially during the formation of pleural effusion. These reports suggested that VEGF plays an important role in the tumor growth of MPM. Strizzi et al.[11] reported that higher VEGF levels were found in the pleural effusions of MPM patients than in those of patients with non-malignant pleural diseases; however, there was no significant correlation between VEGF levels and MPM patient survival. In the present study, we demonstrated that patients with MPM had significantly higher pleural effusion VEGF levels than a population with non-malignant pleuritis or lung cancer involving malignant pleural effusion, and moreover, there was a significant correlation between VEGF levels and MPM patient survival. The discrepancy between our observations and those of the previous study with regard to the correlation between VEGF pleural effusion levels and MPM patient survival may be explained as follows: first, they examined 12 MPM patients; whereas, we examined more patients; second, their MPM patients were classified into IA ($n = 1$), IB ($n = 5$), II ($n = 5$), and III stages ($n = 1$); whereas, our patients included more advanced stages.

Here, we examined the pleural effusion and serum VEGF levels in 16 MPM patients and demonstrated that there was a significant correlation between them. In these patients, 14 patients showed higher VEGF levels in their serum and/or pleural effusion. On the other hand, 12 and 9 patients had higher VEGF levels in their serum and pleural effusion, respectively. Only 2 stage I patients demonstrated lower VEGF levels in both their serum and pleural effusion. So the measurement of VEGF levels in both serum and pleural effusions seems to be an efficient way of diagnosing MPM.

The prognostic significance of VEGF in MPM has been estimated previously.[31] However, the previous study demonstrated a significant correlation between the VEGF staining of resected specimens and short survival. In the present study, we demonstrated that pleural effusion VEGF levels gradually increased according to the progression of the disease, and the Kaplan–Meier method revealed a significant correlation between pleural effusion VEGF levels and survival, which suggested its usefulness as a marker for estimating prognosis.

Conclusion

In summary, we demonstrated that patients with MPM had significantly higher pleural effusion VEGF levels than a population with non-malignant pleuritis involving benign asbestos pleurisy or lung cancer involving malignant pleural effusion, suggesting VEGF to be a useful marker for MPM. The patients with advanced stage MPM showed higher levels of VEGF than the patients with early stage MPM, and the Kaplan–Meier method revealed a significant correlation between pleural effusion VEGF levels and patient survival. Moreover, Cox's regression analysis demonstrated that the pleural effusion VEGF level had an independent statistically significant prognostic effect on survival, which suggested its usefulness as a marker for estimating prognosis.

Acknowledgements

We thank Ms. Hidemi Kitai for providing technical assistance. This work was supported by grants from KAKENHI, a Grant-in-Aid for Scientific Research (C) (20590936), Funds for Cancer Research from the Hyogo Prefecture Health Promotion Association, and Special Coordination Funds for Promoting Science and Technology (H18-1-3-3-1).

Contributors: Tabata C, Tabata R and Nakano T designed research. Tabata C and Hirayama N performed research. Tabata C, Hirayama N, Maeda R, Yasumitsu A, Yamada S, Kuribayashi K and Fukuoka K collected data. Tabata C and Tabata R analyzed and interpreted data. Tabata C performed statistical analysis. Tabata C and Tabata R wrote the manuscript.

Conflicts of interest

We declare that no conflicts of interest exist.

References

1. Robinson BW, Musk AW, Lake RA. Malignant mesothelioma. *Lancet* 2005;**366**:397–408.

2. Robinson BW, Lake RA. Advances in malignant mesothelioma. *N Engl J Med* 2005;**353**:1591—603.
3. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape province. *Br J Ind Med* 1960;**17**:260—71.
4. Selikoff IJ, Hammond EC, Seidman H. Latency of asbestos disease among insulation workers in the United States and Canada. *Cancer* 1980;**15**:2736—40.
5. Vogelzang NJ, Rusthoven JJ, Symanowski J, Sporn MB, Roberts AB. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. *J Clin Oncol* 2003;**21**:2636—44.
6. Nowak AK, Lake RA, Kindler HL, Robinson BW. New approaches for mesothelioma: biologics, vaccines, gene therapy, and other novel agents. *Semin Oncol* 2002;**29**:82—96.
7. Fuhrman C, Duche JC, Chouaid C, Abd Alsamad I, Atassi K, Monnet I, et al. Use of tumor markers for differential diagnosis of mesothelioma and secondary pleural malignancies. *Clin Biochem* 2000;**33**:405—10.
8. Paganuzzi M, Onetto M, Marroni P, Filiberti R, Tassara E, Parodi S, et al. Diagnostic value of CYFRA 21-1 tumor marker and CEA in pleural effusion due to mesothelioma. *Chest* 2001; **119**:1138—42.
9. Tabata C, Tabata R, Kadokawa Y, Hisamori S, Takahashi M, Mishima M, et al. Thalidomide prevents bleomycin-induced pulmonary fibrosis in mice. *J Immunol* 2007;**179**:708—14.
10. Yasumitsu A, Tabata C, Tabata R, et al. Clinical significance of serum VEGF in malignant pleural mesothelioma. J Thorac Oncol, in press.
11. Strizzi L, Catalano A, Vianale G, Orecchia S, Casalini A, Tassi G, et al. Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. *J Pathol* 2001;**193**: 468—75.
12. Rusch VW. A proposed new international TNM staging system for malignant pleural mesothelioma. From the International Mesothelioma Interest Group. *Chest* 1995;**108**:1122—8.
13. Rake C, Gilham C, Hatch J, Darnton A, Hodgson J, Peto J. Occupational, domestic and environmental mesothelioma risks in the British population: a case-control study. *Br J Cancer* 2009;**100**:1175—83.
14. Pass HI, Lott D, Lonardo F, Harbut M, Liu Z, Tang N, et al. Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. *N Engl J Med* 2005;**353**:1564—73.
15. Robinson BW, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, et al. Mesothelin-family proteins and diagnosis of mesothelioma. *Lancet* 2003;**362**:1612—6.
16. Frebourg T, Lerebours G, Delpech B, Bertrand D, Bertrand P, Maingonnat C, et al. Serum hyaluronate in malignant pleural mesothelioma. *Cancer* 1987;**59**:2104—7.
17. Schouwink H, Korse CM, Bonfrer JM, Hart AA, Baas P. Prognostic value of the serum tumour markers cyfra 21-1 and tissue polypeptide antigen in malignant mesothelioma. *Lung Cancer* 1999;**25**:25—32.
18. Creaney J, Yeoman D, Naumoff LK, Hof M, Segal A, Musk AW, et al. Soluble mesothelin in effusions: a useful tool for the diagnosis of malignant mesothelioma. *Thorax* 2007;**62**:569—76.
19. Tabata C, Tabata R, Hirayama N, Yasumitsu A, Yamada S, Murakami A, et al. All- *trans*-retinoic acid inhibits tumor growth of malignant pleural mesothelioma in mice. *Eur Respir J* 2009;**34**:1159—67.
20. Versnel MA, Claesson-Welsh L, Hammacher A, Bouts MJ, van der Kwast TH, Eriksson A, et al. Human malignant mesothelioma cell lines express PDGF beta-receptors whereas cultured normal mesothelial cells express predominantly PDGF alpha-receptors. *Oncogene* 1991;**6**:2005—11.
21. Fitzpatrick DR, Bielefeldt-Ohmann H, Himbeck RP, Jarnicki AG, Marzo AL, Robinson BW. Transforming growth factor-beta: antisense RNA-mediated inhibition affects anchorage-independent growth, tumorigenicity and tumor-infiltrating T-cells in malignant mesothelioma. *Growth Factors* 1994;**11**:29—44.
22. Marzo AL, Fitzpatrick DR, Robinson BW, Scott B. Antisense oligonucleotides specific for transforming growth factor beta2 inhibit the growth of malignant mesothelioma both in vitro and in vivo. *Cancer Res* 1997;**57**:3200—7.
23. Mórocz IA, Schmitter D, Lauber B, Stahel RA. Autocrine stimulation of a human lung mesothelioma cell line is mediated through the transforming growth factor alpha/epidermal growth factor receptor mitogenic pathway. *Br J Cancer* 1994; **70**:850—6.
24. Galffy G, Mohammed KA, Dowling PA, Nasreen N, Ward MJ, Antony VB. Interleukin 8: an autocrine growth factor for malignant mesothelioma. *Cancer Res* 1999;**59**:367—71.
25. Edwards JG, Swinson DE, Jones JL, Muller S, Waller DA, O'Byrne KJ. Tumor necrosis correlates with angiogenesis and is a predictor of poor prognosis in malignant mesothelioma. *Chest* 2003;**124**:1916—23.
26. Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. Vascular-specific growth factors and blood vessel formation. *Nature* 2000;**407**:242—8.
27. Jain RK. Molecular regulation of vessel maturation. *Nat Med* 2003;**9**:685—93.
28. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003;**9**:669—76.
29. Masood R, Kundra A, Zhu S, Xia G, Scalia P, Smith DL, et al. Malignant mesothelioma growth inhibition by agents that target the VEGF and VEGF-C autocrine loops. *Int J Cancer* 2003;**104**:603—10.
30. Li Q, Yano S, Ogino H, Wang W, Uehara H, Nishioka Y, et al. The therapeutic efficacy of anti vascular endothelial growth factor antibody, bevacizumab, and pemetrexed against orthotopically implanted human pleural mesothelioma cells in severe combined immunodeficient mice. *Clin Cancer Res* 2007;**13**:5918—25.
31. Demirag F, Unsal E, Yilmaz A, Caglar A. Prognostic significance of vascular endothelial growth factor, tumor necrosis, and mitotic activity index in malignant pleural mesothelioma. *Chest* 2005;**128**:3382—7.