Increased expression of matrix metalloproteinase 9 and tubulin-α in pulmonary sclerosing hemangioma

SU JIN CHO[1,2], LIAN-JIN JIN[1,2], BU-YEO KIM[4], SU-IN CHO[4], WOON YONG JUNG[1,2], JOUNG-HO HAN[2,3], SEONG-YEON HA[2,5], HAN KYEOM KIM[1,2] and AEREE KIM[1,2]

[1]Department of Pathology, College of Medicine, and [2]Korea Lung Tissue Bank, Korea University, 80 Guro-dong, Guro-gu, 152-703; [3]Department of Pathology, Sungkyunkwan University School of Medicine, 50 Inwol-dong, Kangnam-gu, 135-710 Seoul; [4]Department of Oriental Medicine, Dongshin University, 252 Daeho-dong, Naju, 520-714 Jeonnam; [5]Department of Pathology, Gachon Medical School, 1198 Kuwol-dong, Namdong-gu, 405-760 Incheon, Republic of Korea

Received July 2, 2007; Accepted August 7, 2007

Abstract. Pulmonary sclerosing hemangioma (PSH) is relatively rare and is usually considered a benign tumor because of its slow growth and solitary characteristics. However, several cases with lymph node metastasis have been reported, and its pathogenesis has not been fully elucidated. Three sets of PSH specimens from the Korea Lung Tissue Bank, obtained with IRB approval, were analyzed through the construction of an oligo-microarray that contained about 32,000 genes. The resulting data were confirmed by real-time RT-PCR. Protein expression levels were checked by performing immunohistochemistry (IHC) and immunoblot analysis. In the 3 specimens of PSH tissues, 72 of the 32,000 genes were commonly found up-regulated and 290 were commonly found down-regulated as compared to non-tumor tissues from each patient. Paraffin-embedded tissues from 11 cases were used to confirm the expression of matrix metalloproteinase 9 (MMP-9) and tubulin-α proteins in the non-tumor and PSH tissues via IHC. In addition, the upregulation of protein expression was confirmed by immunoblot analysis. As expected, in all cases MMP-9 and tubulin-α were expressed at significantly higher levels in the PSH than in the non-tumor tissues. This is the first report on a study of the whole genome of PSH. Increased expression of MMP-9 could induce the metastatic ability of PSH and tubulin-α might be responsible for the sclerotic character of this disease. The results of this study will be useful in helping to understand and effectively manage patients suffering from PSH.

Correspondence to: Dr Aeree Kim, Department of Pathology, College of Medicine, Korea University, 80 Guro-dong, Guro-gu, Seoul 152-703, Republic of Korea
E-mail: ark@korea.ac.kr

Key words: pulmonary sclerosing hemangioma, DNA microarray, matrix metalloproteinase 9, tubulin-α, tissue microarray

Introduction

Pulmonary sclerosing hemangioma (PSH) is a relatively rare tumor and is usually considered benign because of its slow growth and solitary characteristics. It is composed of 2 histologically different types of cells, surface cells and round stromal cells, and typically presents as a mixture of 4 patterns: papillary, solid, sclerotic and hemorrhagic. Histopathological diagnosis can be confirmed through the presence of a mixture of the 4 major histological patterns and through the distinctive cellular components (surface-lining cuboidal and stromal-pale tumor cells) found within a single tumor (1-3). Several cases with lymph node metastasis or multifocal lung involvement have been reported (4,5), but did not have a bad prognosis. It is therefore thought that the nature of this tumor is between benign and malignant. The markers of type II pneumocytes, such as thyroid transcription factor 1 (TTF-1), mucin 1 (MUC1), Thomsen-Friedenreich antigen and CD44v6, showed a significant immunostaining pattern (4,6,7) and the ER-ß receptor expression was also increased in PSH (8), although the biological functions of these genes in terms of the pathology of the tumor are not fully understood.

Recent developments in DNA microarray techniques have made it possible to analyze cancer at the molecular level. Using a DNA microarray, the expression of over 10,000 genes can be analyzed in a single experiment. The application of DNA microarrays to the diagnosis and prediction of the prognosis of cancer would greatly enhance the usefulness of the technique in the clinical field. Using this method, we examined PSH specimens and paired non-tumor lung tissues obtained from Korean patients, and attempted to identify the differentially expressed genes that could be significant during the pathogenesis of PSH. The results of the microarray were confirmed by real-time RT-PCR, immunoblot analysis and immunohistochemistry (IHC) using paraffin-embedded tissues.

In this study, we found that expression levels of MMP-9 and tubulin-α proteins were increased in PSH tissues. These findings can help explain the metastatic ability of PSH.

Materials and methods

Patients and study design. Clinical information from the 11 cases is provided in Table I. Frozen sets of PSH and paired non-tumor surrounding lung tissue were available for only 3 cases, numbered 1-3. These 3 sets were supplied by the Korea Lung Tissue Bank (www.korealtb.org) and were subjected to DNA microarray, immunoblot analysis and RT-PCR. The paraffin-embedded tissues of all 11 cases, including cases 1-3, were subjected to tissue microarray (TMA) and IHC. The protein lysates of cases 4 and 5, which were stored in a deep freezer, were used for immunoblot analysis. Fresh samples were collected at the time of surgery and then immediately frozen in liquid nitrogen and stored at -80˚C until use. Informed consents were obtained from the patients for the use of their operated specimens and clinicopathological data for research purposes. We also obtained IRB approval for the use of the PSH case tissues from the Korea Lung Tissue Bank.

Table I. Clinical data of samples.

Case	Gender	Age (years)	Size (cm)	Dominant type
1	F	42	2.0	SO, SC
2	F	60	1.2	SO, H
3	F	30	4.0	P, SO
4	F	45	2.8	SO, SC
5	F	55	4.5	SO, H
6	M	44	2.3	P, H, SC
7	F	63	1.4	SO, H
8	F	62	2.7	SO, P
9	F	58	3.2	SO, H
10	F	56	2.8	P, SC
11	F	24	3.5	P, SC

F, female; M, male; SO, solid; SC, sclerotic; P, papillary; H, hemorrhagic.

DNA microarray. Total-RNA was isolated from the frozen tissues according to the manufacturer's instructions (Qiagen, Valencia, CA) and its quality was confirmed by the ratio of 28S/18S RNA after agarose gel electrophoresis had been performed.

The probe preparation was performed using the 3DNA array detection system according to the manufacturer's protocol (Genisphere, PA), with 20 μg of total-RNA from tumor tissue to derive fluorescently labeled cDNA. RNA from the paired non-tumor tissue was used as a control. The oligo-microarray (Phalanx Biotech Group, Inc.) contained about 32,000 genes which were hybridized and scanned by a ScanArray scanner (Perkin-Elmer, Boston, MA). Primary data from the image files were obtained using Imagene 5.0 (BioDiscovery, CA) and were then normalized as previously described (9). All the array elements in which the fluorescent intensity of each channel was >1.4 x the local background were considered well measured. Ontology analysis was performed using the OntoExpress and Fatigo programs.

Tissue microarray and immunohistochemistry. TMAs were constructed using the tissues of 11 patients who underwent surgery for PSH between 1995 and 2005 at the Korea University Medical Center. Methods of TMA preparation and IHC were the same as those in Jin *et al*'s study (10). All the samples were H&E stained and then reviewed by 2 pathologists. The representative tumor was marked and tissue cores with a diameter of 3 mm were punched and arrayed into a recipient paraffin block using a Kai sterile dermal biopsy punch (Kai Industries, Seki City, Japan). Three sections were cut from the microarray blocks and then transferred to coated slides (Instrumedics, NJ). Immunohistochemical staining was performed using the standard streptavidin-biotin-peroxidase complex method and an automated staining system (Autostainer Plus, Dako, Glostrup, Denmark). Antigen retrieval was performed by microwave oven treatment for 15 min in 0.01 M citrate buffer (pH 6.0). The endogenous peroxidase in the tissue sections was blocked with 3% hydrogen peroxide for 20 min. The slides were then incubated with the appropriately diluted primary antibodies [Monoclonal anti-human MMP-9 antibody, 1:100 (Chemicon International Inc., Temecula CA) Plyclonal Rabbit anti-human tubulin-α 1:100 (Cell Signaling Technology Inc., MA)]. After washing with TBS, the tissue sections were incubated with biotinylated secondary antibody and then with diaminobenzidine substrate, as provided in a Dako Envision kit (Dako). The nuclei were counter-stained with hematoxylin. The slides were then dehydrated, mounted and examined. The images were analyzed using Image-Pro Plus 6.0 (MediaCybernetics Inc., MD).

Immunoblot analysis. The protein lysates (30 μg/lane) were electrophoresed (Bio-Rad, Hercules, CA) on 8% SDS polyacryl amide gel and then transferred to nitrocellulose membranes. The membranes were blocked by incubation in 5% skim milk for 1 h, washed 3 times and then probed with MMP-9 antibody (1:1000 dilution, Chemicon International Inc.) or tubulin-α antibody (1:1000 dilution, Cell Signaling Technology Inc.). After overnight incubation, the membranes were washed 3 times and incubated with secondary antibody solution for 1 h. They were then washed 3 times for 30 min and once with TBS for 5 min. Protein detection and quantitation were carried out using ECL solution (Amersham, Arlington, IL) on X-ray film (Pierce, Rockford, IL).

Results

Pathologic analysis. Clinical information for the patients is described in Table I. More than 2 dominant types were present, but the patterns differed somewhat from each other. In cases 1-3, the solid type was predominantly observed. Frozen sections of PSH and paired non-tumor sets were available only for cases 1-3, and were further used for DNA microarray analysis.

DNA microarray. Differential expression between PSH and non-tumor lung tissue was analyzed using a human oligo-microarray containing about 32,000 genes. Differentially-expressed genes that changed more than 2-fold were identified

Table II. Up-regulated genes in each case.

Case 1		Case 2		Case 3	
Accession no.	Gene	Accession no.	Gene	Accession no.	Gene
NM_000493.2	COL10A1	NM_022097.1	LOC63928	NM_001854.2	COL11A1
NM_022049.1	GPR88	NM_000493.2	COL10A1	NM_000217.1	KCNA1
NM_000640.2	IL13RA2	NM_022336.1	EDAR	NM_018398.2	CACNA2D3
NM_138455.2	CTHRC1	NM_002422.2	MMP3	NM_004004.3	GJB2
NM_015163.4	TRIM9	XM_113916.5	LOC201181	NM_016932.3	SIX2
NM_002373.4	MAP1A	NM_000290.2	PGAM2	NM_000735.2	CGA
BQ011545	PLAC9	NM_021189.2	IGSF4B	NM_000396.2	CTSK
AB018258	ATP10B	NM_147175.2	HS6ST2	NM_138455.2	CTHRC1
NM_018475.2	TPARL	NM_015991.1	C1QA	NM_006207.1	PDGFRL
NM_002421.2	MMP1	NM_000520.2	HEXA	NM_003182.1	TAC1
NM_003020.1	SGNE1	NM_001842.3	CNTFR	XM_374893.2	ZFPL1
NM_000093.2	COL5A1	NM_000868.1	HTR2C	XM_043500.4	LOC92196
NM_145256.2	LRRC25	NM_032219.2	FLJ22269	NM_000793.3	DIO2
NM_015133.2	MAPK8IP3	NM_001200.1	BMP2	NM_000474.2	TWIST1
NM_024038.2	MGC2803	NM_144642.3	SYNPR	NM_000891.2	KCNJ2
NM_014726.1	ProSAPiP2	NM_018670.1	MESP1	NM_004101.2	F2RL2
NM_006475.1	POSTN	NM_001738.1	CA1	NM_144661.2	C10orf82
XM_030300.7	UNC5A	NM_014978.1	SORCS3	NM_001878.2	CRABP2
NM_000845.1	GRM8	NM_003471.2	KCNAB1	NM_015722.2	DRD1IP
NM_004994.1	MMP9	NM_016615.2	SLC6A13	NM_152997.2	C4orf7

Table III. Down-regulated genes in each case.

Case 1		Case 2		Case 3	
Accession no.	Gene	Accession no.	Gene	Accession no.	Gene
NM_020208.2	SLC6A20	BE792494	MGC24381	BM541936	HSPB1
XM_378620.1	LOC283904	NM_005807.1	PRG4	NM_001730.3	KLF5
NM_022475.1	HHIP	NM_006790.1	TTID	NM_152390.1	MGC33926
NM_032933.3	C18orf45	NM_016206.1	FLJ38507	NM_005904.2	SMAD7
NM_178821.1	FLJ25955	NM_016523.1	KLRF1	NM_002776.3	KLK10
NM_002509.2	NKX2-2	NM_001557.2	IL8RB	NM_001874.3	CPM
BE792494	MGC24381	XM_055636.3	KIAA1912	NM_001151.2	SLC25A4
NM_002670.1	PLS1	NM_004235.3	KLF4	NM_152701.2	ABCA13
NM_003015.2	SFRP5	NM_182584.1	FLJ33706	NM_003106.2	SOX2
NM_005310.1	GRB7	AK022159	UBE2E2	NM_002483.3	CEACAM6
NM_031457.1	MS4A8B	NM_007191.2	WIF1	NM_000401.2	EXT2
NM_014787.2	DNAJC6	NM_021992.1	TMSNB	NM_021784.3	FOXA2
NM_000667.2	ADH1A	NM_013377.2	PDZRN4	NM_007072.2	HHLA2
NM_007053.2	CD160	NM_004815.2	PARG1	NM_022351.2	EFCBP1
NM_033393.1	KIAA1727	NM_021139.1	UGT2B4	NM_021242.3	MID1IP1
NM_016206.1	FLJ38507	NM_002013.2	FKBP3	AK074339	RPL7L1
XM_067585.10	LOC131873	NM_152677.1	ZSCAN4	NM_000963.1	PTGS2
NM_199328.1	CLDN8	NM_000550.1	TYRP1	NM_021083.2	XK
NM_013308.2	GPR171	XM_378620.1	LOC283904	NM_001049.2	SSTR1
NM_003843.2	SCEL	NM_001442.1	FABP4	AK055730	SLC23A3

Table IV. Commonly up- or down-regulated genes in all 3 cases.

Up-regulated genes		Down-regulated genes	
Accession no.	Gene	Accession no.	Gene
NM_006207.1	PDGFRL	NM_022475.1	HHIP
NM_004994.1	MMP9	NM_005310.1	GRB7
NM_015136.1	STAB1	NM_014787.2	DNAJC6
NM_001733.2	HAGH	NM_000667.2	ADH1A
NM_006682.1	FGL2	XM_067585.10	LOC131873
NM_152240.1	WIG1	XM_055636.3	KIAA1912
NM_145804.1	ABTB2	NM_001753.3	CAV1
NM_001006612.1	WBP5	NM_005139.1	ANXA3
NM_003486.4	SLC7A5	NM_004392.4	DACH1
NM_002414.3	CD99	NM_000216.1	KAL1
NM_001849.2	COL6A2	NM_000702.2	ATP1A2
NM_000618.2	IGF1	NM_015236.2	LPHN3
NM_002046.2	GAPD	NM_001442.1	FABP4
NM_000308.1	PPGB	XM_297816.5	LOC343637
NM_032704.2	TUBA6	NM_001451.1	FOXF1
NM_144492.1	CLDN14	NM_153371.2	LNX2
NM_004587.1	RRBP1	NM_024913.3	FLJ21986
NM_000597.2	IGFBP2	NM_173505.1	ANKRD29
NM_004766.1	COPB2	NM_001146.3	ANGPT1
NM_001849.2	COL6A2	NM_145650.2	MUC15

as up- or down-regulated genes. There were 564 up-regulated genes in case 1, 792 in case 2 and 1,411 in case 3, with 72 genes in common among them (Tables II and IV). Of the 72 commonly up-regulated genes, 64 could be annotated and 47.37% had a function in protein binding (data not shown). There were 887 down-regulated genes in case 1, 1,043 in case 2 and 1,994 in case 3; 290 genes were held in common among them (Tables III and IV). Of the 290 commonly down-regulated genes, 270 could be annotated and 45.87% of them had a relationship with protein binding (data not shown). Five of the commonly up- or down-regulated genes were selected for further study: MMP-9, collagen type VI, insulin-like growth factor 1 (IGF1) and tubulin-α, which were commonly over-expressed, and mucin 15 (MUC15) , which was commonly under-expressed. MMP-9 was up-regulated 6.7-, 4.6- and 19.4-fold in cases 1-3, respectively. Collagen type VI was up-regulated 3.7-, 2.7- and 12.7-fold in each case, respectively. IGF1 showed 3.0-, 2.1- and 18.5-fold increases and tubulin-α showed 2.0-, 3.2- and 3.5-fold increases in each case, respectively. The MUC15 gene showed a 0.2, 0.2 and 0.11 level of expression in PSH tissues compared to the non-tumor counterparts in cases 1-3, respectively. The gene-expression levels of these 5 selected genes were confirmed by real-time RT-PCR (data not shown).

Confirmation of gene expression via IHC. Expression patterns in the tissue were examined by IHC (Figs. 1 and 2) and analyzed using Image-Pro Plus 6.0. MMP-9 expression was increased by about 34% in PSH tissues compared to paired

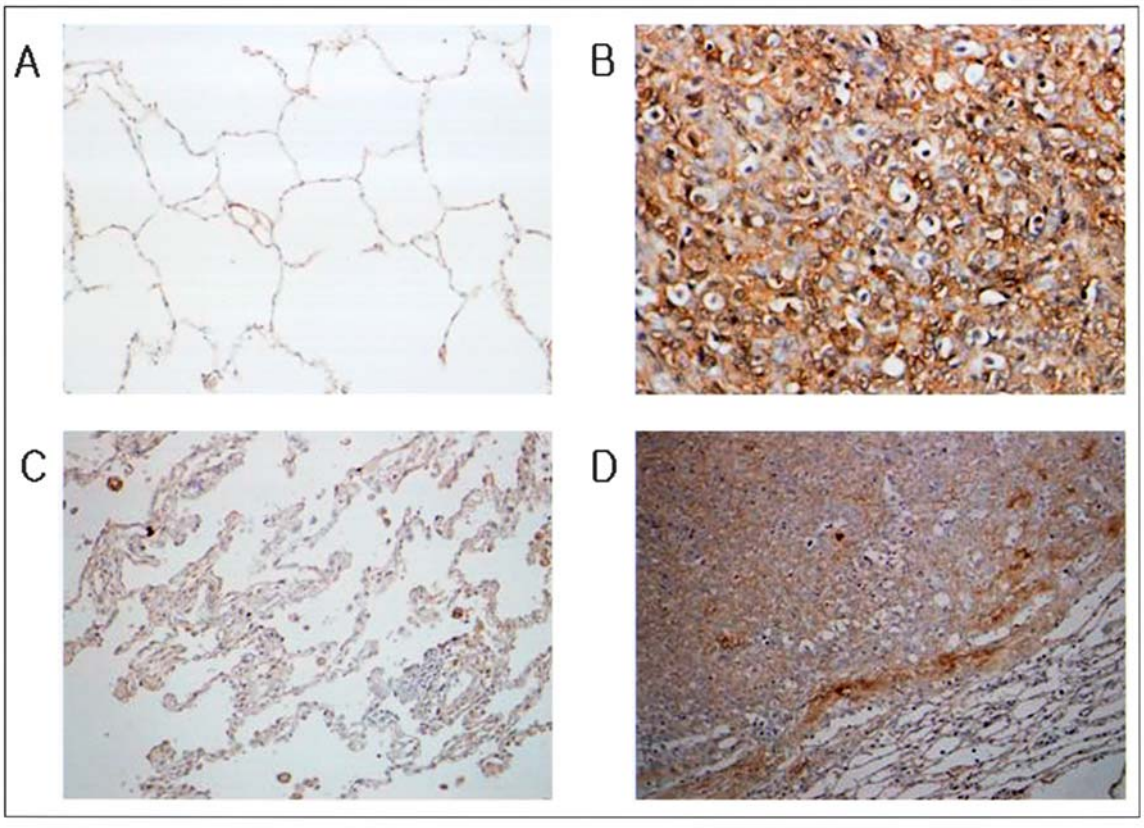

Figure 1. Up-regulated expressions of MMP-9 in pulmonary sclerosing hemangioma tissues. Non-tumor tissues (A,C) and their paired pulmonary sclerosing hemangioma tissues (B,D) (x200).

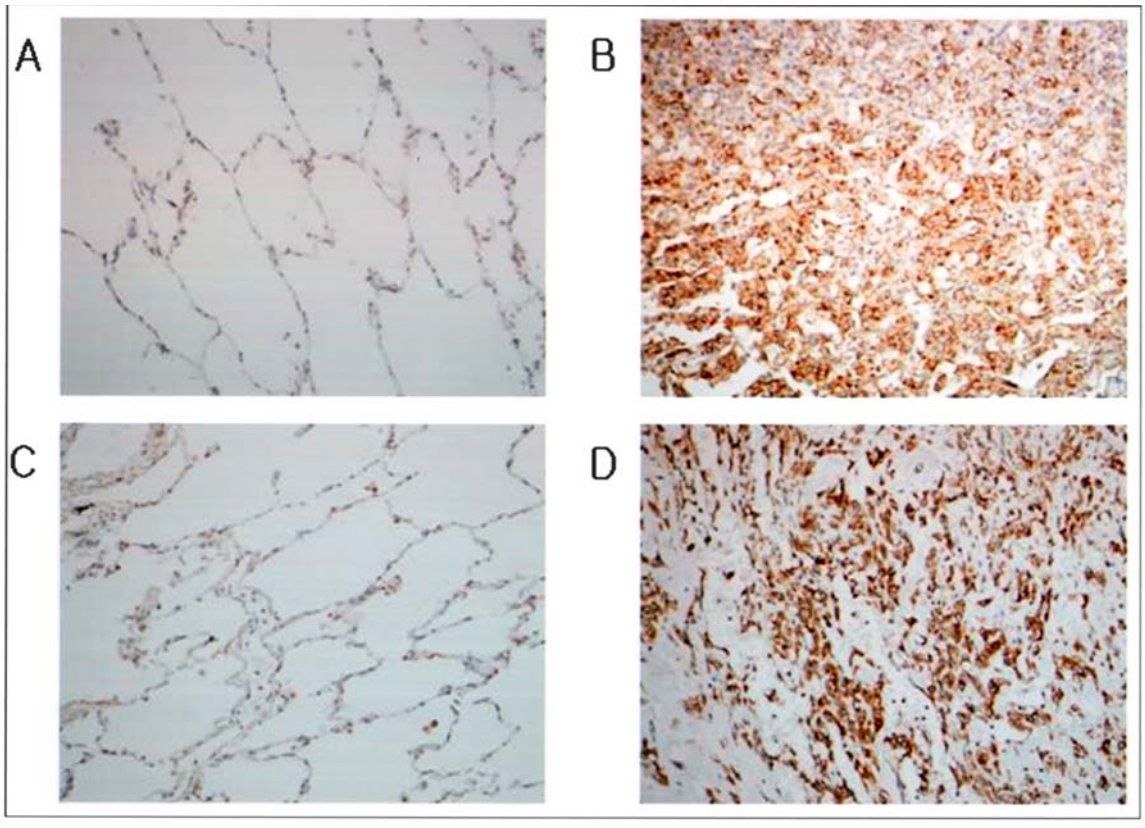

Figure 2. Up-regulated expressions of tubulin proteins in pulmonary sclerosing hemangioma tissues. Non-tumor tissues (A, C) and their paired pulmonary sclerosing hemangioma tissues (B, D) (x200).

Table V. Immunohistochemical data analysis.

MMP-9			Tubulin-α		
Case	Non-tumor	Tumor	Case	Non-tumor	Tumor
1	107.6	122.6	1	81.0	139.0
2	104.0	111.4	2	101.0	133.0
3	91.2	116.8	3	80.0	115.0
4	113.6	140.6	4	88.4	133.6
5	115.4	161.2	5	83.2	130.6
6	92.6	155.8	6	85.2	108.6
7	130.8	150.8	7	81.8	111.2
8	110.8	179.2	8	83.6	148.2
9	104.2	148.0	9	85.2	111.0
10	100.2	115.8	10	74.4	142.6
11	78.8	140.0	11	80.4	106.2
P=0.0003			P=0.000008		

non-tumor tissues (Table V). The expression patterns differed somewhat from case to case. In tumor case 3, MMP-9 expression was up-regulated, especially around the tumor margins. MMP-9 protein was mainly observed in the stroma. Tubulin-α expression was up-regulated by about 50% in PSH tissues as compared to non-tumor tissues (Table V).

Immunoblot analysis. Protein expression levels of MMP-9 and tubulin-α were also confirmed by immunoblot analysis.

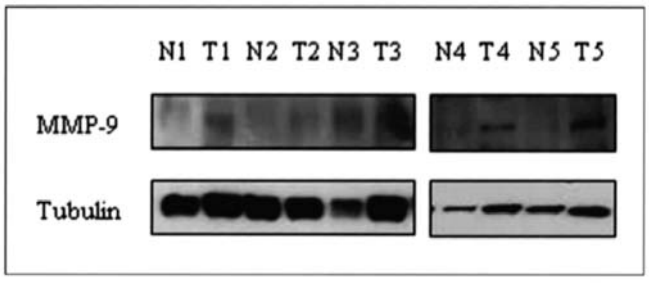

Figure 3. Up-regulations of MMP-9 and tubulin proteins in pulmonary sclerosing hemangioma tissues, as confirmed by immuno-blot analysis. N, non-tumor tissue; T, tumor tissue.

As shown in Fig. 3, MMP-9 protein expression was increased in PSH. An additional 2 sets, cases 4 and 5, were used for immunoblot analysis. MMP-9 expression especially, in cases 1, 4 and 5, was markedly increased. Tubulin-α expression in cases 3 and 4 was also prominently increased.

Discussion

PSH is usually present as a solitary nodule and is diagnosed by its presentation of a mixture of 4 cell pattern types: papillary, solid, hemorrhagic and sclerotic. It is generally considered a benign malady, but several cases with lymph node metastasis or multifocal pulmonary involvement have been reported (4,5). The origin of PSH is controversial; however, recent studies on the expression levels of estrogen receptors and the markers of type II pneumocytes, such as TTF-1, MUC1, Thomsen-

Friedenreich antigen and CD44v6, have shown that PSH is an epithelial neoplasm derived from type II pneumocyte (3,7,8). Because of the rareness of PSH, most studies on it have been case reports, with few being conducted at the genetic level. In our previous study, we identified 10 proteins that were differentially expressed in PSH and non-tumor lung tissues via proteome research (10). DNA microarrays can analyze the expression of over 10,000 genes at the same time, and many kinds of cancer have been analyzed in this way recently (11,12). In this experiment, we used an oligo-microarray that contained about 32,000 genes in order to analyze PSH at the molecular level. Of the genes that are expressed differentially during the development of PSH, we focused on those that may have a relationship with sclerosis, type II pneumocytes or PSH's ability to metastasize.

MMP-9 is a protease that leads to extracellular matrix degradation. It cleaves gelatins, is thought to be involved in matrix remodeling and cell migration, and plays a very important role in cancer development and metastasis. In 2005, Wu *et al* showed that the expression of estrogen receptor ß was increased in PSH (8). Interestingly, it was also reported that estrogen receptor ß increased the invasiveness of breast cancer both *in vivo* and *in vitro* through the up-regulation of MMP-9 (13). Our results were consistent with those of these studies; the expression levels of MMP-9 were elevated in PSH tissues (Figs. 1 and 3). Fig. 1D shows the marked increase of MMP-9 at the tumor margin. Although the tumor samples used in this study were not diagnosed as metastatic, this change in MMP-9 expression could explain the metastatic characteristics of PSH.

IGF1 and its receptor constitute a potent proliferative signaling pathway that stimulates growth and blocks apoptosis in many different cell types. IGF1 stimulates mitogenesis, survival and cellular transformation in an endocrine, autocrine and paracrine fashion (14). It has been reported as increased in prostate cancer, colorectal cancer and lung cancer (14,15), and also provides a mitogenic signal that acts as a growth factor for many types of tissue culture cells. IGF1 associates with tyrosine kinase receptors, such as Shc, Grb2 and Sos-1, to activate Ras and the MAPK cascade (16). Is synthesized during early fetal life, with high concentrations being found in the lung, stimulating lung growth (17). Our study showed that IGF1 was increased during the development of PSH. From this result, we can postulate that IGF1 might encourage the proliferation of lung cells and result in the development of PSH.

Collagen type VI A2 encodes 1 of the 3 α chains of type VI collagen, which is found in most connective tissues, while the cytoskeleton is comprised of tubulin-α6 A2. Collagen type VI A2 binds to extracellular matrix proteins and plays an important role in organizing the matrix components. The functions of these 2 proteins are not exactly understood. However, the known functions of other members of this protein family suggest that they are involved in sclerosis.

Mucin is heavily glycosylated protein that is secreted as massive aggregates of proteins of various sizes. At least 19 human mucin genes have been distinguished. The major secreted airway mucins are MUC5AC and MUC5B. Increased mucin production is observed in lung adenocarcinomas and non-neoplastic lung disease such as asthma, bronchitis or cystic fibrosis. MUC1 acts as a marker for type II pneumocytes, although its function hasn't been fully determined. The role, in this experiment, of the down-regulation of MUC15 during the development of PSH needs further study.

In this study, we show that MMP-9 and tubulin-α proteins are up-regulated during the development of PSH. This is the first report on all the genome levels of PSH. Conducting further studies on the differentially regulated genes obtained by microarray might well explain the pathogenesis of PSH.

Acknowledgements

This study was supported by a Special Research Materials Banks grant from the Ministry of Science and Technology/ Korea Science and Engineering Foundation (KOSEF, R21-2002-000-00015-0). We appreciate the help of Iehwan Bae with the immunohistochemistry.

References

1. Niho S, Suzuki K, Yokose T, Kodama T, Nishiwaki Y and Esumi H: Monoclonality of both pale cells and cuboidal cells of sclerosing hemangioma of the lung. Am J Pathol 152: 1065-1069, 1998.
2. Fukayama M and Koike M: So-called sclerosing hemangioma of the lung. An immunohistochemical, histochemical and ultrastructural study. Acta Pathol Jpn 38: 627-642, 1988.
3. Wang E, Lin D, Wang Y, Wu G and Yuan X: Immunohistochemical and ultrastructural markers suggest different origins for cuboidal and polygonal cells in pulmonary sclerosing hemangioma. Hum Pathol 35: 503-508, 2004.
4. Kim KH, Sul HJ and Kand DY: Sclerosing hemangioma with lymph node metastasis. Yonsei Med J 44: 150-154, 2003.
5. Komatsu T, Fukuse T, Wada H and Sakurai T: Pulmonary sclerosing hemangioma with pulmonary metastasis. Thorac Cardiovasc Surg 54: 348-349, 2006.
6. Yamazaki K: Type-II pneumocyte differentiation in pulmonary sclerosing hemangioma:ultrastructural differentiation and immunohistochemical distribution of lineage-specific transcription factors (TTF-1, HNF-3 alpha, and HNF-3 beta) and surfactan t proteins. Virchows Arch 445: 45-53, 2004.
7. Yoo SH, Jung KC, Kim JH, *et al:* Expression patterns of markers for type II pneumocytes in pulmonary sclerosing hemangiomas and fetal lung tissues. Arch Pathol Lab Med 129: 915-919, 2005.
8. Wu CT, Chang YL and Lee YC: Expression of the estrogen receptor beta in 37 surgically treated pulmonary sclerosing hemangiomas in comparison with non-small cell lung carcinomas. Hum Pathol 36: 1108-1112, 2005.
9. Yang YH, Dudoit S, Luu P, *et al:* Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Res 30: e15, 2002.
10. Jin LJ, Shin BK, Jung WY, *et al:* Proteomic analysis of pulmonary sclerosing hemangioma. Proteomics 6: 4877-4883, 2006.
11. Dasu MR, Barrow RE, Hawkins HK and McCauley RL: Gene expression profiles of giant hairy naevi. J Clin Pathol 57: 849-855, 2004.
12. Olivier RI, van Beurden M and Van' T Veer LJ: The role of gene expression profiling in the clinical management of ovarian cancer. Eur J Cancer 42: 2930-2938, 2006.
13. Hou YF, Yuan ST, Li HC, *et al:* ERß exerts multiple stimulative effects on human breast carcinoma cells. Oncogene 23: 5799-5806, 2004.
14. Grimberg A and Cohen P: Role of insulin-like growth factors and their binding proteins in growth control and carcinogenesis. J Cell Physiol 183: 1-9, 2000.
15. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM and Egger M: Insulin-like growth factor (IGF)-1, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 363: 136-153, 2004.
16. Leahy M, Lyons A, Krause D and O'Connor R: Impaired Shc, Ras, and MAPK activation but normal Akt activation in FL5.12 cells expressing an insulin-like factor I receptor mutated at tyrosines 1250 and 1251. J Biol Chem 279: 18306-18313, 2004.
17. Stiles AD and D'Ercole AJ: The insulin-like growth factors and the lung. Am J Respir Cell Mol Biol 3: 93-100, 1990.