Integrins $\alpha 2\beta 1$, $\alpha 5\beta 1$ and $\alpha v\beta 5$ Are Related to Tumor Growth and Metastasis of Non-small Cell Lung Cancer

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Abstract : **Objective :** Integrins are related to the growth, invasion, and metastasis of cancer cells, and lung cancer cells express multiple integrin subunits. This study was designed to determine the integrin subunits which play major roles in the growth and metastasis of non-small cell lung cancer (NSCLC). **Methods :** NSCLC cell lines EBC-1, LK-2, LC-1F, PC-14, PC-3, VMRC-LCD and IA-5 were transplanted into athymic mice both subcutaneously and intravenously. Subcutaneous tumor sizes were measured twice a week. Carcinoma cells from evident metastatic lesions were obtained and cultured. The patterns of integrin subunit expression were evaluated using immunofluorescence flowcytometric analysis. **Results :** Maximal subcutaneous tumor size in the exponential growth phase was negatively correlated with integrin $\alpha 5$ and $\beta 1$ expression ($p=0.028$, $p=0.035$). Three months after transplantation, carcinoma cells from 11 evident metastatic lesions were obtained and cultured. Expression of integrins $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, αv , $\beta 1$, $\beta 3$, $\beta 4$ and $\beta 5$ was evaluated by flowcytometry. Expression of integrins $\alpha 2$, $\alpha 3$, $\alpha 5$, $\alpha 6$, $\beta 1$, $\beta 4$ and $\beta 5$ was commonly detected in original cell lines. The evident changes at the sites of metastasis were increases in $\alpha 2$ ($p=0.04$), αv ($p=0.05$), $\beta 1$ ($p=0.008$) and $\beta 5$ ($p=0.09$) expression. **Conclusion :** These results suggest that integrins $\alpha 2\beta 1$ and $\alpha v\beta 5$ are related to metastasis, and that $\alpha 5\beta 1$ is related to tumor growth of NSCLC.

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Key words : Flowcytometry, Integrin, Metastasis, Non-small cell lung cancer

Integrins are a superfamily of cell surface adhesion molecules which are mainly concerned with adhesion to extracellular matrix^{1)~4)}. Integrin molecules are heterodimers of an α -subunit and a β -subunit; there are 16 α -subunits and 8 β -subunits included, yielding 22 different heterodimers.

Several integrins play important roles in cancer growth and metastasis. It has been reported that $\alpha v\beta 3$ plays an important role in the angiogenesis of breast cancer, and that inhibition of $\alpha v\beta 3$ causes inability of tumor growth^{5)~7)}. Integrins $\alpha 2\beta 1$ and $\alpha v\beta 3$ are reported to regulate the matrix metalloprotease which promotes invasion of cancer cells⁸⁾⁹⁾. Transfection of $\alpha 4\beta 1$ integrin in CHO

cells gives them the ability to metastasize to bone¹⁰⁾.

It is known that lung cancer cells express several integrin subunits^{11)~13)}. However, it is not known which integrins affect the growth and metastasis of lung cancer. This study was designed to select integrins which play important roles in the growth and metastasis of non-small cell lung cancer (NSCLC).

Materials and Methods

Seven NSCLC cell lines, EBC-1, LK-2, PC-3, VMRC-LCD, LC-1F, PC-14 and IA-5, were transplanted into athymic mice (Balb/c-nu/nu). EBC-1, LK-2, PC-3 and VMRC-LCD were provided by the Japanese Collection of Research Bioresources Cell Bank (Setagaya-Ku, Tokyo, Japan). LC-1F, PC-14 and IA-5 were provided by the Riken Cell Bank (Tsukuba, Ibaragi, Japan). A total of 1×10^6 cells of each cell line were transplanted both subcutaneously and intravenously. Thirty-five four-week-old female athymic mice were used in this study.

Subcutaneous tumor sizes were measured twice a week. Three months after transplantation the mice were sacrificed. Carcinoma cells from evident metastatic lesions were obtained and cultured. After several passages of cul-

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Table 1. Integrin subunit expression of non-small cell lung cancer cell lines

cell line	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$	αv	$\beta 1$	$\beta 3$	$\beta 4$	$\beta 5$
[squamous cell carcinoma]											
EBC-1	n.d.	489.59	4.96	n.d.	0.17	104.17	1.18	57.62	n.d.	36.21	4.26
LK-2	0.22	16.20	0.24	n.d.	0.06	16.55	0.41	30.20	n.d.	1.40	0.63
LC-1F	n.d.	80.80	0.89	1.97	7.60	81.85	n.d.	143.01	n.d.	15.71	n.d.
[adenocarcinoma]											
PC-14	n.d.	247.19	2.45	n.d.	5.34	196.54	1.44	76.92	n.d.	58.77	9.86
PC-3	0.55	22.20	4.11	0.34	2.35	81.74	3.33	98.45	0.40	83.12	2.75
VMRC-LCD	5.18	0.60	0.55	0.26	0.27	5.42	1.67	12.02	n.d.	0.12	0.73
[large cell carcinoma]											
IA-5	0.12	277.67	3.78	0.35	61.84	73.87	1.31	601.65	0.60	0.52	15.66

Values are net-fluorescence intensities. n.d. = not detected

ture, the patterns of integrin subunit expression of the cells were evaluated and compared with those of the original cells using immunofluorescence flowcytometric analysis.

Aliquots (100 μ l) containing 1×10^6 cells were subjected to indirect immunofluorescence staining for expression of surface integrins using a saturated dose of monoclonal antibodies ($\alpha 1$; clone 5E8D9; Upstate Biochemicals, Lake Placid, NY, USA, $\alpha 2$; clone AK7; Pharmingen, San Diego, CA, USA, $\alpha 3$; clone P1B5; Life Technologies Oriental Inc., Nihonbashi, Chuo-Ku, Tokyo, $\alpha 4$; clone SG 73; Seikagaku Corporation, Chuo-Ku, Tokyo, Japan, $\alpha 5$; clone KH72; from Dr K. Miyake, $\alpha 6$; clone GoH3; Immunotech, Cedex, Marseille, France, αv ; clone AMF7; Immunotech, $\beta 1$; clone SG19; Seikagaku Corporation, $\beta 3$; clone SZ21; Immunotech, $\beta 4$; clone AA3; Becton Dickinson, Franklin Lakes, NJ, USA, $\beta 5$; clone P1F6; Immunotech). Control cells (no monoclonal antibody added) were prepared for evaluation of nonspecific fluorescence intensity. The FACS Calibur system (Becton Dickinson) was used for the analysis. The levels of expression of integrin subunits were evaluated using net-fluorescence intensity, which was calculated as the geometric mean fluorescence intensities of stained cells subtracted from that of control cells. Fluorescence intensities of stained cells and those of control cells in each sample were compared using Kolomogorov-Smirnov statistical analysis. When fluorescence intensities of the sample did not reach statistical significance ($p < 0.05$), integrin subunit expression was regarded as negative.

Spearman's rank-correlation coefficient was performed to examine correlation between integrin subunit expression and subcutaneous tumor size. Wilcoxon signed rank test was employed to evaluate the significance of the differences of the integrin subunit expression between cell

Fig. 1. Growth of a Subcutaneous Tumor.

An example of the pattern of growth of a mouse subcutaneous tumor is illustrated. Growth was logarithmic in the initial several weeks.

lines. P values less than 0.05 were taken to indicate statistical significance.

Results

1 : Integrin expression

Integrin subunit expression of the NSCLC cell lines is summarized in Table 1. Expression of integrin $\alpha 2$, $\alpha 3$, $\alpha 5$, $\alpha 6$, αv , $\beta 1$, $\beta 4$ and $\beta 5$ was commonly detected in NSCLC cell lines.

2 : Tumor growth

The subcutaneous tumors exhibited a logarithmic growth pattern for the initial several weeks (Fig. 1). The maximal tumor size of the logarithmic growth phase of each cell line was negatively correlated with the intensity of $\alpha 5$ subunit expression ($p = 0.028$, $R_s = 0.893$, Spearman's rank-correlation coefficient) (Fig. 2). The same correlation was observed for the intensity of $\beta 1$ subunit expression ($p = 0.035$, $R_s = 0.857$). There were no significant corre-

lations between maximal tumor size and the intensities of the other integrin subunits expressions.

Fig. 2. Maximal Subcutaneous Tumor Size and Integrin $\alpha 5$ Expression.

Maximal subcutaneous tumor sizes in exponential growth phase were negatively correlated with net-fluorescence intensities of the integrin $\alpha 5$ subunit ($p=0.028$). Y axis error bars shows standard error of tumor size.

3 : Metastasis

Carcinoma cells from 11 evident metastatic lesions were obtained 3 months after transplantation. Net-fluorescence intensities of integrin subunits of these 11 cell lines from metastatic lesions and their metastatic sites are summarized in Table 2. Net-fluorescence intensities of integrin $\alpha 2$ ($p=0.04$), αv ($p=0.05$), $\beta 1$ ($p=0.008$) and $\beta 5$ ($p=0.09$) subunits were increased at the sites of metastasis compared with those in original cells (Wilcoxon signed rank test).

Discussion

Cancer cells derived from each organ have their own integrin expression pattern. The distinctive features of NSCLC, compared with normal lung tissue, are a lack of $\alpha 1$, an increase in $\alpha 2$, and a decrease in $\alpha 5$ expression¹⁴⁾¹⁵⁾. The NSCLC cells we used commonly expressed integrin subunits $\alpha 2$, $\alpha 3$, $\alpha 5$, $\alpha 6$, αv , $\beta 1$, $\beta 4$ and $\beta 5$. This feature of integrin subunit expression may be related to the malignant characteristics of NSCLC such as spontaneous growth and metastasis. However, there has been no direct

Table 2. Change in integrin subunit expression at sites of metastasis

cell-line	metastasis	$\alpha 2$	$\alpha 3$	$\alpha 5$	$\alpha 6$	αv	$\beta 1$	$\beta 4$	$\beta 5$
[squamous cell carcinoma]									
EBC-1	original	489.59	4.96	0.17	104.17	1.18	57.62	36.21	4.26
	cervical lymphnode	576.36 ↑	4.51 ↓	0.11 ↓	77.44 ↓	n.d. ↓	123.76 ↑	32.47 ↓	3.65 ↓
	cervical lymphnode	472.78 ↓	4.54 ↓	0.03 ↓	95.30 ↓	2.21 ↑	88.63 ↑	42.67 ↑	4.39 ↑
LK-2	original	16.20	0.24	0.06	16.55	0.41	30.20	1.40	0.63
	leg bone	25.99 ↑	0.41 ↑	0.83 ↑	24.28 ↑	2.06 ↑	54.4 ↑	2.17 ↑	5.09 ↑
	adrenal gland	19.05 ↑	0.17 ↓	0.31 ↑	19.22 ↑	2.06 ↑	38.17 ↑	1.29 ↓	2.06 ↑
	skull	19.44 ↑	0.98 ↑	0.70 ↑	18.35 ↑	2.74 ↑	34.88 ↑	1.34 ↓	10.51 ↑
LC-1F	original	80.80	0.89	7.60	81.85	n.d.	143.01	15.71	n.d.
	vertebral bone	84.25 ↑	2.08 ↑	15.84 ↑	100.24 ↑	1.25 ↑	187.55 ↑	15.98 ↑	2.56 ↑
[adenocarcinoma]									
PC-14	original	247.19	2.45	5.34	196.54	1.44	76.92	58.77	9.86
	cervical lymphnode	244.29 ↓	2.36 ↓	1.20 ↓	82.49 ↓	1.60 ↑	61.55 ↓	36.81 ↓	4.14 ↓
	brain	423.53 ↑	1.96 ↓	6.07 ↑	164.55 ↓	0.69 ↓	134.37 ↑	78.02 ↑	7.32 ↓
VMRC-LCD	original	0.60	0.55	0.27	5.42	1.67	12.02	0.12	0.73
	vertebral bone	1.14 ↑	0.33 ↓	0.89 ↑	14.17 ↑	2.66 ↑	32.18 ↑	0.19 ↑	4.92 ↑
[large cell carcinoma]									
IA-5	original	277.67	3.78	61.84	73.87	1.31	601.65	0.52	15.66
	adrenal gland	749.01 ↑	5.18 ↑	132.77 ↑	122.19 ↑	1.62 ↑	1194.62 ↑	1.05 ↑	28.39 ↑
	adrenal gland	391.01 ↑	5.35 ↑	52.35 ↓	67.45 ↓	1.99 ↑	675.70 ↑	2.83 ↑	24.92 ↑
increased (number of cell-lines)		9	5	7	6	9	10	7	8
decreased (number of cell-lines)		2	6	4	5	2	1	4	3
		$p = 0.04$	n.s.	n.s.	n.s.	$p = 0.05$	$p = 0.008$	n.s.	$p = 0.09$

Values are net-fluorescence intensities. n.d. = not detected ↑ : higher than for original cells ↓ : lower than for original cells

experimental research on the correlation between integrin levels and cancer growth and metastasis of NSCLC.

To perform quantitative analysis of integrin expression, we used flowcytometric analysis of cultured single cells. Because it is difficult to induce metastasis of human NSCLC cell lines in athymic mice, we used both subcutaneous and intravenous inoculation of cancer cells.

In this study, maximal subcutaneous tumor size was negatively correlated with expression of integrin $\alpha 5$ and $\beta 1$. Integrin $\alpha 5\beta 1$ expression appeared to be one of the biological factors affecting tumor growth of NSCLC. However, in our study integrin $\alpha 5\beta 1$ expression affected none of in vivo growth rates of tumors, in vitro growth rates, and in vitro saturation densities. The levels of other integrin subunits exhibited no correlation with maximal subcutaneous tumor size. Juliano has already reported that the decrease in integrin $\alpha 5\beta 1$ expression in CHO cells is correlated with subcutaneous tumor growth in athymic mice¹⁶⁾¹⁷⁾. It was suggested that extracellular matrix recognition by the $\alpha 5\beta 1$ integrin plays a role in the control of proliferation of CHO cells. Probably the same mechanism exists in NSCLC cells. Many other biological factors affect cell proliferation, such as tumor suppressor gene status, oncogene status, and growth factor receptors. However, we did not investigate such factors in this study.

There have been many investigations reported concerning integrins and cancer metastasis. The metastatic capacity of integrin $\alpha 4\beta 1$ - and $\alpha 6\beta 1$ -lacking lymphoma cells is greatly reduced¹⁸⁾. It has been reported that invasion and metastasis of prostatic cancer cells are related to increase in $\alpha 6\beta 4$ integrin and decrease in $\alpha 3\beta 1$ integrin expression¹⁹⁾. Integrin $\alpha L\beta 2$ plays an important role in the pro-

cess of liver metastasis in malignant lymphoma cells²⁰⁾. Matsuura reported that transfection of the $\alpha 2\beta 1$ integrin in RD cells gave them the capacity to metastasize to multiple organs²¹⁾. Transfection of $\alpha 4\beta 1$ integrin in CHO cells gave them the capacity to metastasize to bone¹⁰⁾.

Since patterns of integrin expression are different for each cancer cell line, the above findings do not apply to all cancer cells. For example, NSCLC often metastasizes to bone. However, since integrin $\alpha 4\beta 1$ expression of NSCLC cells was mostly negative in this study, this integrin does not appear to play a role in the process of NSCLC bone metastasis.

Chen reported that a clonotype with a high level expression of $\alpha 2\beta 1$ displays a substantial increase in experimental engraftment and metastasis of human NSCLC cells in SCID mice¹⁴⁾. Integrin $\alpha 2\beta 1$ is the receptor for type I collagen and plays a role in regulation of matrix metalloprotease, which in turn plays an important role in cancer cell invasion via adhesion to collagen. Integrin $\alpha v\beta 5$ is the receptor for vitronectin and has been reported to play a role in migration and dissemination of cancer cells²²⁾²³⁾.

In our study the only evident changes in integrin expression at sites of metastasis were increases in $\alpha 2\beta 1$ and $\alpha v\beta 5$. This result suggests that integrins $\alpha 2\beta 1$ and $\alpha v\beta 5$ play roles in the process of metastasis of NSCLC cells.

In this study, NSCLC cells commonly expressed integrin subunits $\alpha 2$, $\alpha 3$, $\alpha 5$, $\alpha 6$, αv , $\beta 1$, $\beta 4$ and $\beta 5$. Decreases in $\alpha 5\beta 1$ expression in NSCLC cells may be important in the growth of tumors. Integrins $\alpha 2\beta 1$ and $\alpha v\beta 5$ may play roles in the process of metastasis of NSCLC.

References

- 1) Hynes RO : Integrins : a family of cell surface receptors. *Cell* 48 : 549-54, 1987.
- 2) Hynes RO : Integrins : versatility, modulation, signaling in cell adhesion. *Cell* 69 : 11-25, 1992.
- 3) Hemler ME : VLA proteins in the integrin family : structures, functions, and their role on leukocytes. *Annu Rev Immunol* 8 : 365-400, 1990.
- 4) Stepp MA, Spurr Michaud S, Tisdale A, et al : Alpha 6 beta 4 integrin heterodimer is a component of hemidesmosomes. *Proc Natl Acad Sci USA* 87 : 8970-4, 1990.
- 5) Brooks PC, Montgomery AM, Rosenfeld M, et al : Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 79 : 1157-64, 1994.
- 6) Brooks PC, Clark RA, Cheresh DA : Requirement of vascular integrin alpha v beta 3 for angiogenesis. *Science* 264 : 569-71, 1994.
- 7) Brooks PC, Stromblad S, Klemke R, et al : Antiintegrin alpha v beta 3 blocks human breast cancer growth and angiogenesis in human skin. *J Clin Invest* 96 : 1815-22, 1995.
- 8) Riikonen T, Westermarck J, Koivisto L, et al : Integrin alpha 2 beta 1 is a positive regulator of collagenase (MMP-1) and collagen alpha 1 (I) gene expression. *J Biol Chem* 270 : 13548-52, 1995.
- 9) Brooks PC, Stromblad S, Sanders LC, et al : Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. *Cell* 85 : 683-93, 1996.
- 10) Matsuura N, Puzon-McLaughlin W, Takada Y, et al : Induction of experimental bone metastasis in mice by transfection of integrin alpha 4 beta 1 into tumor cells. *Am J Pathol* 148 : 55-61, 1996.

- 11) Hirasawa M, Shijubo N, Uede T, et al : Integrin expression and ability to adhere to extracellular matrix proteins and endothelial cells in human lung cancer lines. *Br J Cancer* 70 : 466-73, 1994.
- 12) Mette SA, Pilewski J, Buck CA, et al : Distribution of integrin cell adhesion receptors on normal bronchial epithelial cells and lung cancer cells in vitro and in vivo. *Am J Respir Cell & Molecular Biol* 8 : 562-72, 1993.
- 13) Falcioni R, Cimino L, Gentileschi MP, et al : Expression of beta 1, beta 3, beta 4, and beta 5 integrins by human lung carcinoma cells of different histotypes. *Exp Cell Res* 210 : 113-22, 1994.
- 14) Chen FA, Repasky EA, Bankert RB : Human lung tumor-associated antigen identified as an extracellular matrix adhesion molecule. *J Exp Med* 173 : 1111-9, 1991.
- 15) Koukoulis GK, Warren WH, Virtanen I, et al : Immunolocalization of integrins in the normal lung and in pulmonary carcinomas. *Hum Pathol* 28 : 1018-25, 1997.
- 16) Giancotti FG, Ruoslahti E : Elevated levels of the alpha 5 beta 1 fibronectin receptor suppress the transformed phenotype of Chinese hamster ovary cells. *Cell* 60 : 849-59, 1990.
- 17) Schreiner CL, Fisher M, Juliano RL, et al : Defective vasculature in fibronectin-receptor-deficient CHO cell tumors in nude mice. *Int J Cancer* 55 : 436-41, 1993.
- 18) Stroecken PJ, van Rijthoven EA, van der Valk MA, et al : Targeted disruption of the beta1 integrin gene in a lymphoma cell line greatly reduces metastatic capacity. *Cancer Research* 58 : 1569-77, 1998.
- 19) Dedhar S, Saulnier R, Nagle R, et al : Specific alterations in the expression of alpha 3 beta 1 and alpha 6 beta 4 integrins in highly invasive and metastatic variants of human prostate carcinoma cells selected by in vitro invasion through reconstituted basement membrane. *Clin Exp Metastasis* 11 : 391-400, 1993.
- 20) Harning R, Myers C, Merluzzi VJ : Monoclonal antibodies to lymphocyte function-associated antigen-1 inhibit invasion of human lymphoma and metastasis of murine lymphoma. *Clin Exp Metastasis* 11 : 337-42, 1993.
- 21) Chan BMC, Matsuura N, Takada Y, et al : In vitro and in vivo consequences of VLA-2 expression on rhabdomyosarcoma cells. *Science* 251 : 1600-2, 1991.
- 22) Doerr ME, Jones JI : The roles of integrins and extracellular matrix proteins in the insulin-like growth factor I-stimulated chemotaxis of human breast cancer cells. *J Biol Chem* 271 : 2443-7, 1996.
- 23) Brooks PC, Klemke RL, Schon S, et al : Insulin-like growth factor receptor cooperates with integrin alpha v beta 5 to promote tumor cell dissemination in vivo. *J Clin Invest* 99 : 1390-8, 1997.