Original article

Integrins α2β1, α5β1 and αvβ5 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 α5 and β1 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 α1, α2, α3,α4, α5, α6, αv, β1, β3, β4 and β5 was evaluated by flowcytometry. Expression of integrins α2,α3,α5,α6,β1,β4 and β5 was commonly detected in original cell lines. The evident changes at the sites of metastasis were increases in α2 (p=0.04), αv (p=0.05), β1 (p=0.008) and β5 (p =0.09) expression. **Conclusion** : These results suggest that integrins α2β1 and αvβ5 are related to metastasis, and that α5β1 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 αvβ3 plays an important role in the angiogenesis of breast cancer, and that inhibition of αvβ3 causes inability of tumor growth[5)～7)]. Integrins α2β1 and αvβ3 are reported to regulate the matrix metalloprotease which promotes invasion of cancer cells[8)9)]. Transfection of α4β1 integrin in CHO cells gives them the ability to metastasize to bone[10)].

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	α1	α2	α3	α4	α5	α6	αv	β1	β3	β4	β5
[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

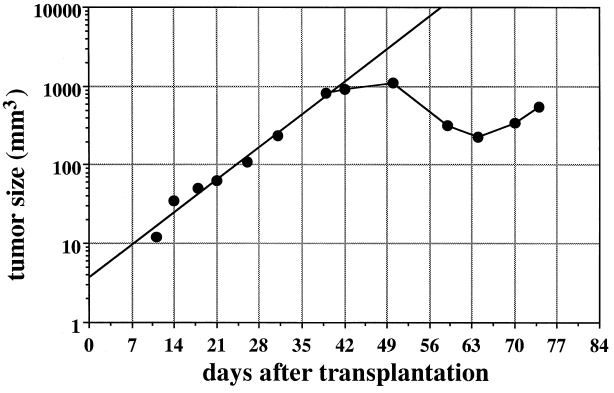
Values are net-fluorescence intensities. n.d. = not detected

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 (100μl) containing 1×10^5 cells were subjected to indirect immunofluorescence staining for expression of surface integrins using a saturated dose of monoclonal antibodies (α1 ; clone 5E8D9 ; Upstate Biochemicals, Lake Placid, NY, USA, α2 ; clone AK7 ; Pharmingen, San Diego, CA, USA, α3 ; clone P1B5 ; Life Technologies Oriental Inc., Nihonbashi, Chuo-Ku, Tokyo, α4 ; clone SG73 ; Seikagaku Corporation, Chuo-Ku, Tokyo, Japan, α5 ; clone KH72 ; from Dr K. Miyake, α6 ; clone GoH3 ; Immunotech, Cedex, Marseille, France,αv ; clone AMF7 ; Immunotech,β1 ; clone SG19 ; Seikagaku Corporation, β3 ; clone SZ21 ; Immunotech,β4 ; clone AA3 ; Becton Dickinson, Franklin Lakes, NJ, USA, β5 ; 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 lines. P values less than 0.05 were taken to indicate statistical significance.

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.



Results

1 : Integrin expression

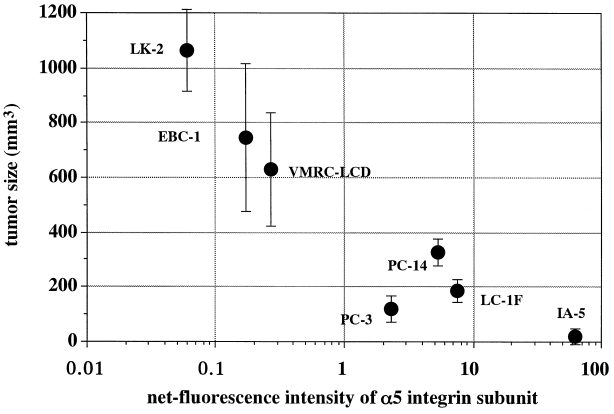
Integrin subunit expression of the NSCLC cell lines is summarized in Table 1. Expression of integrin α2, α3, α5, α6, αv, β1, β4 and β5 was commonly detected in NSCLC cell lines.

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 α5 subunit expression ($p=0.028$, $Rs=0.893$, Spearman's rank-correlation coefficient) (Fig. 2). The same correlation was observed for the intensity of β1 subunit expression ($p=0.035$, $Rs=0.857$). 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 α 5 Expression.
Maximal subcutaneous tumor sizes in exponential growth phase were negatively correlated with net-fluorescence intensities of the integrin α5 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 α 2 (p=0.04), αv (p=0.05), β1 (p=0.008) and β5 (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 α 1, an increase in α2, and a decrease in α5 expression[14)15)]. The NSCLC cells we used commonly expressed integrin subunits α2, α3, α5, α6, αv, β1, β4 and β5. 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	α2	α3	α5	α6	αv	β1	β4	β5
[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 α5 and β 1. Integrin α5β1 expression appeared to be one of the biological factors affecting tumor growth of NSCLC. However, in our study integrin α5β1 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 α5β1 expression in CHO cells is correlated with subcutaneous tumor growth in athymic mice[16)17)]. It was suggested that extracellular matrix recognition by the α5β1 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 α4β1- and α6β1-lacking lymphoma cells is greatly reduced[18)]. It has been reported that invasion and metastasis of prostatic cancer cells are related to increase in α6β4 integrin and decrease in α3β1 integrin expression[19)]. Integrin αLβ2 plays an important role in the process of liver metastasis in malignant lymphoma cells[20)]. Matsuura reported that transfection of the α2β1 integrin in RD cells gave them the capacity to metastasize to multiple organs[21)]. Transfection of α4β1 integrin in CHO cells gave them the capacity to metastasize to bone[10)].

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 α4β1 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 α2β1 displays a substantial increase in experimental engraftment and metastasis of human NSCLC cells in SCID mice[14)]. Integrin α2β1 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 αvβ5 is the receptor for vitronectin and has been reported to play a role in migration and dissemination of cancer cells[22)23)].

In our study the only evident changes in integrin expression at sites of metastasis were increases in α2β1 and αvβ5. This result suggests that integrins α2β1 and αvβ5 play roles in the process of metastasis of NSCLC cells.

In this study, NSCLC cells commonly expressed integrin subunits α2, α3, α5, α6, αv, β1, β4 and β5. Decreases in α5β1 expression in NSCLC cells may be important in the growth of tumors. Integrins α2β1 and αvβ5 may play roles in the process of metastasis of NSCLC.

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