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Alterations of Integrin Expression in Human Lung Cancer

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Integrins are cell-surface receptors which are involved in cell-matrix and/or cell-cell adhesion. They have been suggested to play a role in tumor invasion and metastasis. We examined the expression of various integrin subunits in normal and cancer cells of the lung using 33 human lung cancer cell lines as well as 6 lung cancer samples from which tumor cell lines could be established. This study clearly demonstrated that changes in the expression of certain integrins occur frequently in lung cancer, especially in small cell lung cancer. Loss of the $\alpha 1$ subunit of the β_1 integrin family appears to be the most prominent change, although loss of other integrin subunits such as α_2 or emergence of some integrin subunits such as α_v can also be observed. These results suggest that changes in integrin expression may contribute to the invasive and/or metastatic behavior of lung cancer.

Key words: Integrin — Adhesion molecule — Lung cancer — Metastasis — Invasion

Integrins are a family of cell-surface receptors which are expressed as a heterodimer composed of a single β subunit noncovalently linked to one of several α subunits.[1)] Three integrins with β_1, β_2 and β_3 subunits have been extensively studied, while three additional integrins (β_4, β_5, β_p) have recently been identified. Integrins are known to be involved in cell-matrix and cell-cell adhesion, and are thought to play a role in fundamental cellular processes such as immune responses and platelet aggregation. In addition, some evidence suggests that the processes of tumor invasion and metastasis require a complex coordinated set of changes in cell-matrix and cell-cell interactions.[1,2)]

Recent molecular biological studies have revealed that the accumulation of genetic changes in both dominant and recessive oncogenes is probably necessary for lung carcinogenesis,[3)] but very little is known about the mechanisms of tumor invasion and metastasis of this fatal cancer, which is the leading cause of cancer deaths in the USA and the second-leading cause in Japan.[4,5)] In an effort to gain insight into the possible role of adhesion molecules in the tumor invasion and metastasis of human lung cancer, we examined the expression of integrins in normal and cancer cells of the lung. Profound alterations of integrin expression in lung cancer cells were observed when compared with normal cells of the respiratory tract, suggesting that such changes may contribute to the acquisition of invasive and metastatic phenotypes of lung cancer cells.

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MATERIALS AND METHODS

Monoclonal antibodies Anti-integrin monoclonal antibodies (MoAbs) used in this study were as follows: β_1, K20[6)]; β_2, BL5[7)]; β_3, SZ21[8)]; α_1, TS2/7[9)]; α_2, Gi9[10)]; α_3, J143[11)]; α_4, HP2/1[12)]; α_5, SAM1[13)]; α_6, GoH3[14)]; α_v, AMF/7.[15)] TS2/7 and J143 were generous gifts of Dr. J. Strominger and Dr. L. Old, respectively. The remaining MoAbs were purchased from Immunotech S.A. (Marseilles, France).

Lung cancer cell lines and normal lung tissues Thirty-three lung cancer cell lines analyzed in this study included 17 small cell lung cancer (SCLC) and 16 non-small cell lung cancer (NSCLC) cell lines (7 adenocarcinoma, 6 squamous cell carcinoma and 3 large cell carcinoma). Seventeen SCLC and five NSCLC cell lines with the prefix ACC-LC- were established at Aichi Cancer Center. NCI-H460 was obtained from the American Type Culture Collection (Rockville, MD). The remaining cell lines were kindly provided by various investigators. Derivation and culture conditions have already been reported.[16,17)]

Normal and lung cancer tissues were obtained from surgical specimens. Tissues were embedded in OCT compound (Ames, Elkhart, IN), quickly frozen in ethanol-dry ice as described previously and stored at $-70^{\circ}C$ until analysis.

Flow cytometric analysis Aliquots (100 μl) containing 1×10^6 cells were subjected to indirect immunofluorescence staining for the expression of surface integrins using various MoAbs (10 μg/ml) and were analyzed by FACStar (Becton Dickinson, Mountain View, CA) as described previously.[18)] The expression levels of in-

tegrin subunits in lung cancer cell lines were scored as follows: −, <10%; +, 11–34%; ++, 35–74%; +++, >75%.

Immunohistological staining Four-μm-thick frozen sections of normal and tumor samples were fixed with 4% paraformaldehyde in 0.1 *M* NaH_2PO_4 with 8% sucrose (pH 7.4) for 30 min on ice and immunohistological staining was performed by the avidin-biotin-peroxidase complex (ABC) method as described previously.[19]

RESULTS

Expression of integrins in human normal lung tissues The expression of various integrin subunits in human normal lung tissues was first examined by the ABC method to evaluate differences in the integrin expression between normal and cancer cells of the lung. Our findings are summarized in Table I and representative results are shown in Fig. 1. The β_1 chain was expressed abundantly in epithelial cells of the bronchus, bronchial gland, and alveolar septa. In contrast, the β_2 and β_3 subunits were not found in epithelial cells of the lung.

Strong expression of the β_1 subunit led us to examine expression of various α subunits (α_{1-6} and α_v) which are known to form heterodimers with β_1 subunit.[1] In the epithelial cells of the bronchus, expression of the α_1, α_2, α_3 and α_6 subunits was clearly demonstrated. The α_1 subunit was abundantly expressed in all layers of the bronchial epithelium. In contrast, α_6 was detected exclusively in the region of cell contact with the basement membrane, while the α_3 expression was not confined to the basal cells of the bronchial epithelial cells, but rather was most intense in the area contacting the basement membrane. No expression of the α_4, α_5 or α_v subunits was observed. The bronchial gland was found to express the β_1 subunit and all the α subunits except for α_4 and α_v. Alveolar cells were positive for α_1 and α_3 expression, although other known β_1 integrins were not found.

Expression of integrins in human lung cancer cell lines To detect quantitatively integrins expressed specifically on the cell surface, flow cytometric analysis was carried out using MoAbs specific for various α and β subunits (Tables II and III). β_1 was expressed in all cell lines, while β_2 and β_3 were virtually absent on the cell surface of lung cancer cells, reflecting findings for the expression of integrins in normal lung tissues.

We then examined all α subunits (α_{1-6} and α_v) which can form heterodimers with β_1 subunits (Tables II and III). In contrast to the strong expression of α_1 observed in normal lung, the α_1 subunit was expressed in only 2 of 17 (12%) SCLC cell lines and in 3 of 16 (19%) NSCLC cell lines. Similarly but less frequently, α_2, α_3 and α_6 were absent from neoplastic cells of the lung, although these subunits were positive in normal bronchial epithelium. In other word, these adhesion molecules were expressed in only 35–53% of SCLC and in 69–88% of NSCLC cell lines. In contrast, the α_5 and α_v subunits, which are generally not found in normal lung, were expressed in a considerable fraction of lung cancer cell lines (12–59% of SCLC and 50–75% of NSCLC cell lines). Expression of α_4 was not detected among any of the lines tested except two, reflecting the negative results of staining in normal lung tissues. No significant differences in integrin expression were observed among three major histological subtypes of NSCLC.

Three pairs of SCLC cell lines established consecutively from three individuals were also examined to evaluate whether any changes in integrin expression occurred during the course of this disease (Table II). Increases or decreases in the level of integrin expression were observed occasionally, but no specific pattern for such changes could be found.

Six tumor samples from which lung cancer cell lines could be established were also examined immunohistologically to evaluate whether integrin expressions *in vitro* in lung cancer cell lines faithfully reflect those

Table I. Integrin Expression in Human Normal Lung by the ABC Method

Epithelial cells	β subunit			α subunit						
	β_1	β_2	β_3	α_1	α_2	α_3	α_4	α_5	α_6	α_v
Bronchial epithelium	++[a]	−	−	++	+	+[b]	−	−	+[c]	−
Bronchial gland	++	−	−	+	+	++	−	+[d]	+	−
Alveolar epithelium	++	−	−	+	−	+	−	−	−	−

a) Staining intensity was scored as −, negative; +, positive; ++, strongly positive.
b) Although all layers of the bronchial epithelium showed positive staining, greater intensity was observed in the area of contact with the basement membrane.
c) Positive staining was observed at the region of contact with the basement membrane.
d) Some bronchial glands were weakly positive.

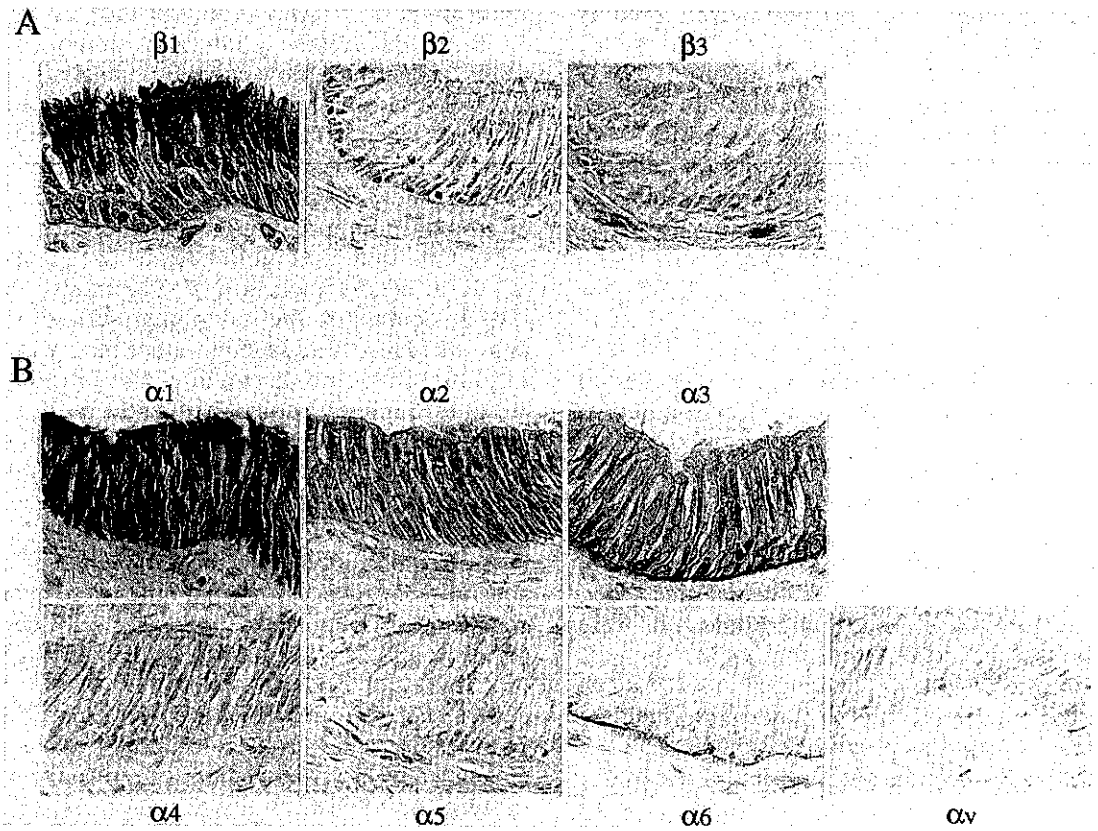

Fig. 1. Expression of integrins in human normal bronchial epithelium. Immunoperoxidase staining was performed using various MoAbs against β_{1-3} (A) and α_{1-6} and α_v (B) integrin subunits by the ABC method. A, β_1 subunit is highly expressed in the normal bronchial epithelium in contrast to the absence of β_2 and β_3. B, All layers of the bronchial epithelium are positive for the expression of α_1 and α_2. In contrast, α_6 is detected exclusively in the region of cell contact with the basement membrane, while α_3 expression is not confined to but is most intense at the area of basement membrane contact. No expression of the α_4, α_5 and α_v subunits is observed.

in vivo in the corresponding tumor samples. Tumor samples from which ACC-LC-5, ACC-LC-60, ACC-LC-67, ACC-LC-93, ACC-LC-94 and ACC-LC-97 had originated were analyzed by the ABC method using all the anti-integrin MoAbs employed in this study (Fig. 2). Integrin expressions in lung cancer tissues were similar to those in the corresponding cell lines with one exception, i.e., moderate expression of α_3 subunit was observed only in tumor tissue of Case 93 but not in the corresponding cell line, ACC-LC-93. These results indicate that changes in the integrin expression observed in *in vitro*-cultured cell lines appear to reflect changes *in vivo* in the corresponding tumor samples.

DISCUSSION

Integrins are expressed in various types of human cancers and have been suggested to play a role in tumor invasion and metastasis.[1,2] Lung cancer cells have also been shown to express certain members of this superfamily,[20-23] although very little is known about their expression pattern in normal lung tissues. Feldman *et al.* observed uniform expression of α_3/β_1 integrin in 5 SCLC cell lines,[23] while Zylstra *et al.* reported α_2/β_1 expression in 4 of 4 NSCLC cell lines.[20] In this study, we analyzed cell-surface expression of various integrin subunits in 33 human lung cancer cell lines as well as those in 6 corresponding tumor tissues. Integrin expression in normal lung was also evaluated to aid interpretation of the results of this study.

We found that the β_1 integrin subunit was strongly expressed in human normal lung, while α_{1-3} and α_6 were positive among α subunits in the β_1 integrins. It is interesting that human lung cancer cell lines showed significant alterations in integrin expression, including the loss of integrins normally expressed in the lung or emergence of certain integrin expressions that are not present in the normal repertory. The most prominent change observed

Table II. Integrin Expression on the Cell Surface of Small Cell Lung Cancer Cell Lines by Flow Cytometric Analysis

Cell lines	β subunit			α subunit						
	β_1	β_2	β_3	α_1	α_2	α_3	α_4	α_5	α_6	α_v
ACC-LC-5	+++[a]	−	−	−	++	−	−	−	++	+
ACC-LC-36[b]	++	−	−	−	+	−	−	+	−	++
ACC-LC-48[b]	+++	−	−	+	+	++	−	−	+	−
ACC-LC-51[b]	+++	−	−	−	+	+	−	−	−	+
ACC-LC-52[b]	+++	−	−	−	−	−	−	−	+	−
ACC-LC-60	+++	−	−	−	−	+	−	−	+	−
ACC-LC-61	+++	−	−	−	−	−	−	−	+	−
ACC-LC-66	+++	−	−	−	−	++	−	−	−	+
ACC-LC-67	+++	−	−	−	++	++	−	−	−	+
ACC-LC-76	++	−	−	−	−	−	−	−	−	++
ACC-LC-80	++	−	−	−	−	+	−	−	++	+
ACC-LC-87[b]	+++	−	++	−	−	+++	−	−	−	+++
ACC-LC-96	+	−	−	−	−	−	−	−	−	−
ACC-LC-97	++	−	−	−	−	++	−	−	−	−
ACC-LC-173[b]	+++	−	+	−	−	+++	−	−	−	+
ACC-LC-175	+++	−	−	+	+	−	−	−	−	+
ACC-LC-177	+++	−	−	−	−	−	++	+	+	−

a) Expression level of integrin subunits was scored as −, <10%; +, 11–34%; ++, 35–74%; +++, >75%.
b) Three pairs of consecutively established cell lines from three individuals were also evaluated: ACC-LC-36 and -51 from patient TM; ACC-LC-48 and -52 from patient OT; ACC-LC-87 and -173 from patient NT.

Table III. Integrin Expression on the Cell Surface of Non-small Cell Lung Cancer Cell Lines by Flow Cytometric Analysis

Cell lines	β subunit			α subunit						
	β_1	β_2	β_3	α_1	α_2	α_3	α_4	α_5	α_6	α_v
Adenocarcinoma										
ACC-LC-93	++[a]	−	−	−	−	−	−	−	−	−
ACC-LC-94	+++	−	−	−	++	+++	+	+++	+	−
ACC-LC-180	+++	−	−	+++	−	−	−	−	+	+
SK-LC-1	+++	−	−	+	+	+++	−	−	−	+
SK-LC-4	+++	−	−	−	+++	+++	−	+	++	+
SK-LC-5	+++	−	−	+	+++	+++	−	+	−	++
SK-LC-7	+++	−	−	−	+	+	−	+	+++	+
Squamous cell carcinoma										
ACC-LC-73	+++	−	−	−	−	+	−	++	++	++
Calu-1	+++	−	−	−	++	+++	−	++	++	++
PC-1	++	−	−	−	−	+	−	−	−	+
PC-10	+++	−	−	−	++	++	−	−	++	−
QG56	+++	−	−	−	+++	+++	−	−	++	++
SK-MES-1	+++	−	−	−	+++	+++	−	+	++	−
Large cell carcinoma										
ACC-LC-91	+++	−	−	−	−	+++	−	−	+	+
Calu-6	+++	−	−	−	++	++	−	−	+	+
NCI-H460	+++	−	−	−	++	++	−	+	+	+

a) Expression level of integrin subunits was scored as −, <10%; +, 11–34%; ++, 35–74%; +++, >75%.

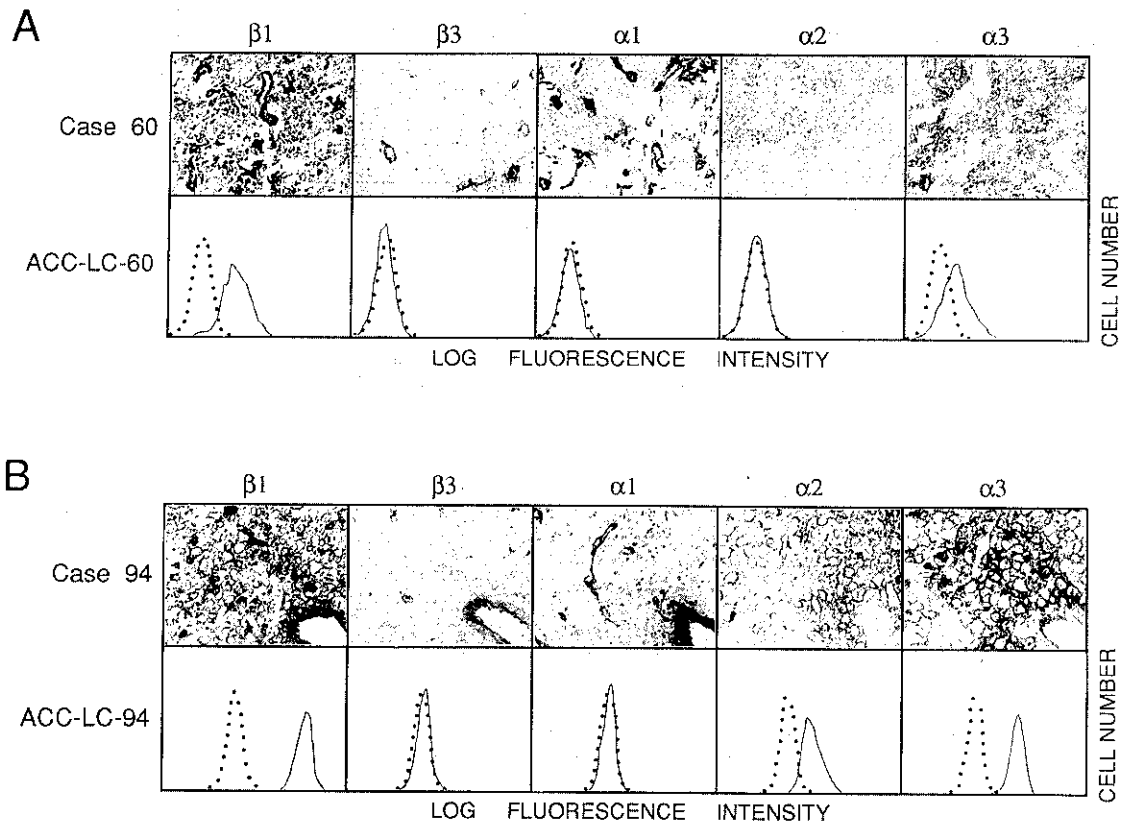

Fig. 2. Expression of integrins in SCLC (A) and NSCLC (B) tumor samples as well as the corresponding cell lines. A, Case 60 as well as the corresponding tumor cell line, ACC-LC-60, showed expression of β_1 and α_3 subunits at high and low levels, respectively. In contrast, β_3, α_1 and α_2 were not detected either *in vivo* or *in vitro*, although integrin expressions were observed in some non-tumorous cells such as blood vessels. B, Abundant expression of β_1 and α_3 subunits were observed in tumor cells of Case 94 as well as the corresponding cell line, ACC-LC-94, while α_2 subunit was expressed at a moderate level. Virtually no expression of β_3 or α_1 subunit was observed in tumor cells *in vivo* or *in vitro*.

in this study was the loss of α_1 expression in lung cancer cells (88% of SCLC and 81% NSCLC cell lines). In addition, a significant fraction of lung cancer cell lines also lost expression of α_2 or α_3. It should be noted that such a loss of integrin expression was observed more frequently in SCLC than in NSCLC, suggesting a possible relation with the morphological and biological differences between these two major histological types of lung cancer. In contrast to α_{1-3} integrins, expressions of α_5 and α_v were found to be upregulated in lung cancer.

Although identifying alterations of integrin expression in lung cancer has been hampered by a lack of knowledge about the expression of the adhesion molecules in normal lung, similar findings have recently been reported in other types of human cancer. Decreased expression of α_2/β_1 in breast cancer was reported by Zutter *et al.*,[24] while Koukoulis *et al.* showed both loss and aberrant expression of various integrins in breast cancer when compared with normal breast tissues.[25] A correlation between reduced expression of α_2/β_1 and higher Dukes' stage has also been reported in colon cancer.[26] In an experimental system, Plantefaber and Hynes reported that significant changes in integrin expression occurred upon oncogenic transformation in rodent cells, suggesting a possible role in acquiring the malignant phenotype.[27] α_1/β_1, α_2/β_1 and α_3/β_1 are known to function as both collagen and laminin receptors, while α_3/β_1 also binds to fibronectin.[1] The loss of these integrins, which may function to restrain cancer cells at sites of tumor formation by binding to components of extracellular matrices, would play a key role in conferring the invasive and metastatic phenotypes upon lung cancer cells. One could also argue that the aberrant upregulation of α_5 and α_v in lung cancer would favor anchoring tumor cells at sites of metastasis.

The present study clearly demonstrated the existence of complex alterations of integrin expression in lung

cancer. Although it seems unlikely that any single member of the integrin superfamily is responsible for the invasive and metastatic behavior of this fatal cancer, diminished expression of certain integrins such as α_1 as well as increased expression of α_5 and α_v, probably all contribute to such malignant behavior in differing degrees. Further studies will be necessary to elucidate whether any correlation exists between the altered integrin expression and the clinical behavior of lung cancer.

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