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ORIGINAL ARTICLE

Expression profiling of integrins in lung cancer cells

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ABSTRACT

Integrins are heterodimeric transmembrane receptors consisting of 18 α and 8 β subunits. Heterodimer composition of α and β subunits has a potential for determining tumor subtypes of human lung cancer. The purpose of this study was to investigate the expression profile of integrins in lung cancer cells. Expression profiling of integrins in a panel of lung cancer cell line, including A549 (adenocarcinoma, ADC), Calu-1 (squamous carcinoma, SCC), H1650 (bronchioloalveolar carcinoma, BAC) and DMS-53 (small cell lung cancer, SCLC), was analyzed by cDNA microarrays, restriction analysis of gene expression (RAGE) and flow cytometry. Seventy-nine lung cancer specimens were used to further validate the data from cell lines using immunohistochemistry. Integrins are obviously expressed in a cell type-specific manner, such as α3 in A549, Calu-1 and H1650 except in DMS53, α4 in H1650, α5 and β1 in all cell lines. The integrins detected with cDNA microarrays were all unequivocally detected with RAGE and by flow cytometry at the protein level. In all lung cancer specimens, α3 integrin was strongly expressed in ADC, SCC and BAC, but was infrequent in SCLC. α4 integrin was solely expressed in BAC. α5 and β1 integrins were expressed in all four histological types of lung cancer specimens. Integrin α3 and α4 may be useful as diagnostic markers for adenocarcinoma, squamous cell carcinoma and bronchioloalveolar carcinoma. RAGE is a promising technique for studying the expression profiles of genes, such as integrins in cancer cells.

Introduction

Integrins are well-known mediators in the attachment of cells to each other and to their surrounding extracellular matrix (ECM). Owing to their cell membrane localization and their dual function, integrins not only preserve tissue integrity by connecting the intracellular actin cytoskeleton with the ECM but also mediate signals for the control of diverse cell functions, including survival, proliferation, differentiation, adhesion and migration [1–3]. So far, 18 α and 8 β subunits of integrins have been identified, which covalently bind to form 24 different transmembrane heterodimers [4]. Each heterodimer is composed of a single α and a single β subunit, and both subunits participate in ligand recognition. Specificity of an integrin in interacting with extracellular ligands is determined by heterodimer composition of α and β subunits. For example, α3β1 integrin predominantly recognizes a receptor for laminin 5, whereas αVβ3 is reported mainly as a ligand of fibronectin and vitronectin and α2β1 as a collagen receptor [4–7].

Lung cancer is one of the most common malignant tumors with more aggressive and high metastatic potential. Evidence now suggests that these features may be due to important links between the cancer cells and the ECM in their local environment [8,9]. Low activation state of integrins on cancer cells is thought to account for the highly metastatic and motile behavior. Investigations have shown that down-regulation of α3 integrin subunit may contribute to the enhanced tumorigenicity of c-myc-overexpressing small cell lung carcinoma, while the loss of αv expression is correlated with the recurrence in node-negative lung carcinoma [8,9]. Increased expression of α1β1 and α2β1 integrins has been shown to be positively correlated with the increased metastatic ability in squamous cell carcinoma [10]. Reduced α3β1 integrin expression is probably related to the increased aggressiveness and a poor factor of prognosis in patients either with small cell lung cancer (SCLC) or non-small cell lung cancer [10,11]. For β1 integrin, it has also been reported that its mediated adhesion of SCLC cells to ECM proteins promotes tyrosine phosphorylation. This blocks chemotherapy-induced activation of the caspase pathway and, hence, apoptosis [13]. Thus, it appears that integrins are potential targets for specific diagnosis and therapy of lung cancer. It will then be necessary to delineate the expression profile of integrins expressed on a particular type of cancer in order to identify a unique target.

In this study, we investigated for the first time the profiling expression of integrins in a panel of lung cancer cell lines using cDNA microarray analysis. Furthermore, we developed a novel method for restriction analysis of gene expression (RAGE) to confirm the results from cDNA microarray analysis.

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Materials and methods

Cell cultures

Cell lines of human lung cancers, including A549 (adenocarcinoma, ADC), Calu-1 (squamous carcinoma, SCC), H1650 (bronchioloalveolar carcinoma, BAC) and DMS-53 (SCLC), were obtained from American Tissue Culture Collection (ATCC). Cells were grown in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin (GIBCO).

RNA extraction

RNAs were extracted from the cell lines using standard techniques. The total RNA was isolated by TRIzol (Invitrogen) according to the manufacturer's instructions, and the integrity of RNA was assessed using an Agilent BioAnalyzer 2100 (Agilent, Palo Alto, CA).

Gene expression microarrays

A pathway-specific gene chip for human extracellular matrix and adhesion molecules (SuperArray Inc. Bethesda, MD) was used to evaluate the expression profile of integrins of each cell line. The microarray membrane contained oligonucleotides complimentary to cDNA encoding integrins of α1–α11, αL, αM, αV, αX and β1 to β8, as well as house-keeping genes (glyceraldehydes-3-phosphate dehydrogenase (GADPH), cyclophilin A, ribosomal protein L13a (RPL13A) and β-actin). Microarray analysis was performed according to the manufacturer's protocol. Briefly, the microarrays were hybridized in labeled cDNA and synthesized by RT-PCR in a 10 μl reaction containing α-^{32}P-dCTP (10 Ci/μl), Rnase inhibitor (40 U/μl), reverse transcriptase (50 U/μl) (Promega, Madison, WI) and GEAprimer mix and labeling buffer provided with the kit. After prehybridization for 2 h, the membrane was hybridized with the total labeled reaction mixture overnight with continuous agitation at 60 °C according to the manufacturer's instructions. After washing, the membrane was exposed to a phosphor screen, scanned in a phosphoimager (Molecular Imager FX, Bio-Rad, Hercules, CA) and the image was analyzed with the software of ScanAlyze2 (Lawrence Berkeley National laboratories) and GEArrayAnalyzer (Super Array, Bethesda, MD). The relative level of expression of each integrin was calculated as a percentage of the total, detectable integrin.

Restriction analysis of gene expression

The results of cDNA microarrays were validated with restriction analysis of gene expression. The procedures are briefly described as below: (1) Reverse transcription was performed with a 50 μl reaction mixture consisting of 25 μg of RNA, 1 mM dNTP and 10 units of avian myeloblastosis virus (AMV) reverse transcriptase (Promega) at 25 °C for 10 min, followed by 42 °C for 2 h. The reaction was stopped by heating at 75 °C for 5 min; (2) design of primers: The homologous regions of either α or β integrins were aligned, and primers were designed using the software of ClustalW of MacVector (Genetics Computer Group, Madison, WI.). For integrins, primer pairs, each consisting of 25–26 bases in length, were designed to cover the homologous β propeller regions of 5–7, extending from 5′-GEQIGSY to LNLDGFT-3′, and to produce amplicons with a size ranging from 250 to 330 base pairs. For β integrins, primer pairs were designed to cover the homologous I-like domain, extending from 5′-RIGFGSF to AIMQ-VAV-3′, and to produce amplicons of 231–249 base pairs. For each primer, 6 extra bases were added to the 5′ end to facilitate amplification during subsequent cycles of PCR. The sequences of primers are provided as supporting materials for this publication. Using the software program of Restriction Enzyme Analysis of MacVector, it has also been predicted that each amplicon yielded fragments with sizes unique to each integrin subunit after digestion with a chosen set of restriction enzymes (Tables 1 and 2) (3) PCR: PCR was performed with 25 μCi of each γ^{33}P-labeled sense primer, 400 nM of each antisense primer, 200 nM dNTP and 25 ng cDNA in a 50 μl reaction mixture. Each reaction mixture was heated to 94 °C for 10 min to denature cDNA, and 1 unit of Taq polymerase was then added (Promega) while the mixture was cooled to 60 °C. The following PCR conditions had been determined to be optimal: 94 °C for 40 s, 50 °C for 90 s and 72 °C for 15 s for 5 cycles, followed by 94 °C for 45 s, 58 °C for 90 s and 72 °C for 20 s for 19 cycles. The PCR products were purified in a 2.4% agarose gel with 0.5 μg/ml of ethidium bromide, followed by extraction with a QIACEX II kit (QIAGEN,) and determination of radioactivity. (4) Restriction digestion: the PCR products were separately digested with Acc I, Ava II, Bsl I, BstN I, Hinf I, Taq I and Alu I for α integrins and with Alu I, Hinf I, Bsp1286 I and Bsl I for β-integrins at the optimal temperature for 45 min (New England Biolabs, Inc). Each sample was then analyzed by electrophoresis with a 7% denaturing polyacrylamide gel. Molecular marker of one

Table 1
List of 24 integrins expression profiles in lung cancer cell lines.

Rank	Gene name	Signal intensity			
		A549	Calu-1	H1650	DMS-53
1	Integrinα1	5.6	5.4	5.0	5.7
2	Integrin α2	7.0	8.2	6.0	5.6
3	Integrin α2B	4.9	4.0	4.8 4.1	
4	Integrin α3	8.3*	8.6*	9.5*	2.5
5	Integrin α4	2.2	2.7	7.9*	1.8
6	Integrin α5	3.8	5.5	6.0	5.7
7	Integrin α6	6.2	7.2	6.8	8.6
8	Integrin α7	5.8	5.5	4.5	4.8
9	Integrin α8	5.7	5.2	4.6	5.3
10	Integrin α9	6.1	5.7	4.4	5.7
11	Integrin α10	5.6	4.8	4.5	4.9
12	Integrin α11	5.8	4.6	4.2	4.9
13	Integrin αL	4.6	4.1	4.3	4.1
14	Integrin αM	5.0	4.6	4.6	4.7
15	Integrin αV	4.1	7.7	7.7	6.0
16	Integrin αX	6.4	5.1	4.9	5.5
17	Integrin β1	42.2	39.0	40.5	27.5
18	Integrin β2	7.7	7.1	8.6	10.0
19	Integrin β3	7.1	7.6	8.7	9.9
20	Integrin β4	6.2	7.6	8.3	9.2
21	Integrin β5	6.4	6.1	9.3	7.9
22	Integrin β6	4.4	5.5	7.5	11.3
23	Integrin β7	5.1	6.6	8.1	9.7
24	Integrin β8	5.0	15.4	9.0	10.6

* >2.0-fold difference.

Table 2
Validation of integrins expression in lung cancer cell by RAGE.

Rank	Gene name	Restriction enzymes		Signal intensity			
		Ava II	Bsp1286 I	A549	Calu-1	H1650	DMS-53
1	ITGα3	69		5.0*	5.7*	5.4*	1.1
2	ITGα4	45		0.9	0.9	4.9*	0.9
3	ITGα5	104		14.7	15.8	15.0	10.2
4	ITGβ1		128	41.3	40.1	43.8	37.4

* >2.0-fold difference.