Establishment of Cell Lines with High and Low Metastatic Potential from A549 Human Lung Adenocarcinoma

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This article reports the establishment of variant cell lines with high and low metastatic potential by the dilution plating technique. Two clones with high metastatic potential, 2S Lu-4 and 11S Lu-1 and two clones with low metastatic potential, 8S and 16S were established from A549 human lung adenocarcinoma. The high-metastatic cell lines produced enhanced lung metastases, but the low-metastatic cell lines did not produce lung metastasis after injection into the tail vein of 5-week-old BALB/c nude mice. The primary tumors produced by the two high-metastatic cells grew fast and showed enhanced angiogenesis. The high-metastatic cells were small and flat-shaped, while the low-metastatic cells were large and flat-shaped. When the four variant cell lines and original A549 cells were embedded in collagen gels, the colonies of 2S Lu-4, 11S Lu-1 and A549 grew actively, whereas almost of all the colonies of 8S and 16S did not survive after 35 days in culture. These four cloned cell lines originated from heterogeneous populations of the parental A549 cells should be an excellent tool for studying the process of metastasis of lung cancer.

Key words: Human lung adenocarcinoma A549 — Establishment of cell lines — Metastasis

Lung carcinoma is one of the most common human cancers and is a predominant cause of cancer-related death throughout the world.1) Despite advances in surgical techniques and the development of aggressive adjuvant therapies for treatment of primary neoplasms, many lung cancers are not curable due to metastases.1, 2) Cancerous tumors contain heterogeneous populations of cells with different biological properties, including different potentials for invasion and metastasis.2)–4) The process of metastasis consists of selection and sequential steps that include angiogenesis, detachment, motility, invasion of the extracellular matrix, intravasation, circulation, adhesion, extravasation into the organ parenchyma and growth.4) The outcome of metastasis depends on the continuous interaction of metastatic cells with host factors, and the ability of tumor cells to produce metastasis is due to a unique set of properties. Multiple genes that regulate different steps in the lung metastatic process have been identified. Several oncogenes and tumor suppressor genes, such as ras, myc, p53, RB and allelic loss of chromosomes play very important roles.5–8) But, it is likely that other genes in addition to these genes are also involved in the process of metastasis of lung cancer. The identification of those unknown genes associated with metastasis is important in order to understand the genotypic and phenotypic characteristics of the metastatic lung cancer cells, and this requires a dependable model system. Namely, the cells should be stable with regard to their metastatic potential, and a number of clones with high and low metastatic potential from the same origin should be available. We have already established and characterized high-metastatic cells, Lu-2, Lu-7, Lu-4, Lu-1 and Lu-5, and low-metastatic cells, 7S, 3S, 8S and 13S from PC-14 human lung adenocarcinoma.9) Furthermore, we isolated a novel actin-related gene, the Arp11 gene, which was specifically expressed in low-metastatic cell lines, 7S, 3S, 8S and 13S by using the mRNA differential display method.10) However, establishment of cell lines with high and low metastatic potential from other cell lines is very important to compare the cellular and molecular basis of high and low metastatic potentials. Therefore, we tried to establish variant cell lines with high and low metastatic potentials from A549 human lung adenocarcinoma. In this article we describe the establishment of two clones with high metastatic potential and two clones with low metastatic potential, and the evaluation of the metastatic and tumorigenic behavior and morphology of these cloned cell lines.

MATERIALS AND METHODS

Mice For all experiments, 5-week-old female athymic BALB/c nude mice were supplied by Charles River Laboratories (Tokyo). Sterile food and water were fed to the mice. The mice were maintained in a sterile environment at constant temperature and humidity.

Tumor lines A549 human lung adenocarcinoma, initiated from a human alveolar cell carcinoma, was kindly provided by Dr. J. D. Minna, the University of Texas, Southwestern Medical Center.11) A549 was used to establish variant cell lines with high and low metastatic potential.
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**Conditions of culture** A549 and variant cells with different metastatic potential from A549 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM medium) (Nisshin Pharmaceutical Co., Tokyo) supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 units/ml) and streptomycin (100 µg/ml) in a highly humidified atmosphere of 5% CO₂ at 37°C. The cell lines were subcultured once or twice a week as required.

**Selection of cell lines with high and low metastatic potential from A549** Cells from parental A549 cells were cloned by a technique based on the dilution plating principle. Single cell suspensions were seeded into 96-well tissue culture plates (Nippon Becton Dickinson Co., Ltd., Tokyo) at 1 cell/well. Wells containing a single cell were identified under a light microscope (×100 magnification) and marked. Colonies were transferred after sufficient growth to vessels of increasing size to expand the cell population. The cloned subpopulations obtained at 3 weeks after cloning were divided into multiple samples. Cells of the fifteen samples were suspended in serum-free DMEM 1640 medium and 5×10⁵ cells/0.2 ml were injected into the tail vein of the nude mice using 27 gauge needles as described by Fidler. Mice were killed when signs of illness were noted. All experimental groups contained at least four mice. At autopsy, single colonies in lung and lymph nodes were removed, minced finely, and plated on tissue culture dishes in culture medium to select the cell lines with high metastatic potential from A549. Subsequently, cloned cells were injected into the tail vein of nude mice. To select low-metastatic cell lines, mice were killed at 141 days after injection into the tail vein of the nude mice and it was confirmed that there was no metastasis in the lung, lymph nodes and other organs. The survival period of mice injected with A549 cells and cloned cell lines with different metastatic potential at 5×10⁵ cells/0.2 ml (n=4 or 5) into the tail vein was evaluated. Mice surviving at 160 days were killed.

**Experimental metastasis assay** The metastatic potential of A549 cells and variant cell lines with high and low metastatic potential derived from the original A549 cells was evaluated by quantitative lung colony assay, as described by Fidler et al. Cells (5×10⁵ cells/0.2 ml, n=4 or 5) were injected into the tail vein of mice. Mice were killed at 58–79 days for the cell lines with high metastatic potential, and 120 days for the cell lines with low metastatic potential after cell inoculation. The number of lung colonies was counted using a magnifying glass.

**Morphology** The morphology of A549 cells and cloned cell lines with different metastatic potential was examined microscopically.

**Tumorigenicity in nude mice and histopathological examination** The tumorigenicity of A549 cells and cloned cell lines with different metastatic potential was examined by injection of 1×10⁷ cells/0.2 ml into the subcutis of the mice (n=5). Tumor growth was monitored three times a week. Tumor volume (V) was calculated by using the formula V=1/2x length x width². For histopathological examination, mice were killed at 50 days after inoculation, and tumors were trimmed, fixed with 10% formalin, and stained with hematoxylin and eosin.

**Collagen embedded culture and dispersion index** Type I collagen stock solution (Cell Matrix Type IA, Nitta Gelatin, Osaka) was mixed with 5× concentrated DMEM and a reconstituting buffer (0.05 N NaOH solution containing 2.2% NaHCO₃ and 200 mM HEPES) to achieve 2 mg/ml final concentration of collagen, according to the manufacturer’s instructions. Fetal bovine serum (FBS) was then added to this mixture to a final concentration of 10%, and 0.5 ml of this solution was plated on a 6 cm plate as the basal layer. After polymerization at 37°C for 30 min, 2 ml of the same collagen solution containing 5×10⁴/ml was overlaid on the basal collagen layer, and again allowed to polymerize at 37°C for 30 min. Finally, 3 ml of DMEM supplemented with 10% FBS was added to the top of collagen gels, and the cells were cultured.

The colonies were observed at 2 weeks in culture. After 25 days, the number of dispersed colonies per total of 50 colonies was counted. For each cell line, four plates were used for the count and statistical analysis. Statistical significance was calculated by using Student’s t test.

**RT-PCR** Cells at 60–80% confluency were harvested and subjected to RNA isolation using a FAST Track mRNA Isolation Kit (Invitrogen, Tokyo) according to the manufacturer’s recommendations. Aliquots (30 ng) of poly(A)+ RNA isolated from A549 and the 4 cell lines were reverse transcribed with oligo dT₁₅ as a primer, and subjected to polymerase chain reaction (PCR) using 5'-TGTTGAGTTCATGGATGTC (sense) and 5'-TTGTGCTTGTTGAGGAACT (antisense) for vascular endothelial growth factor (VEGF), 5'-AGCCACTGTTAAGTGACA (sense) and 5'-ATAGGCTTCTGAGGCAAATG (antisense) for VEGF-B, 5'-AGGCCACTGCTTATGCAA (sense) and 5'-TAGACATGTCATTAAACCT (antisense) for VEGF-C, 5'-CATCTCAGTCCACATTTGG (sense) and 5'-GGCAAGCAGCTTACAACCT (antisense) for VEGF-D, and 5'-CCTTAAATGTCAGCGCGGA (antisense) and 5'-GGGTCAGAAGTGTTTCAAGG (sense) for ß-actin (corresponding to nucleotides 187–206, 463–482, 301–318, 640–657, 511–528, 992–1011, 604–621, 1070–1087, 208–225 and 690–707, respectively; DDBJ/Genbank/EMBL accession numbers, VEGF-B, XM006539; VEGF-C, NM005429; VEGF-D, NM004469; ß-actin, XM037239). The products were fractionated on 0.7% agarose gel and stained with ethidium bromide. PCR conditions were 35 cycles at 94°C for 60 s, 55°C for 120 s, and 72°C for 180 s.

**Homozygous deletion** High-molecular-weight DNA was obtained from A549 cells, cloned cell lines, and the human
small cell lung carcinoma cell line N417 according to the previously described method.29) Information on the DNA markers D9S1749, p16/CDKN2A and D9S171 was obtained from the Genome Database (http://gdbwww.gdb.org/gdb/). The oligonucleotides used to detect the D9S1749 marker were 5'-AGGAGAGG-GTAGCGTTCGAA and 5'-TACAGGGTGCGGGTCA-GATAA, and 5'-AGCTAAGTGACCTCATCTCGTCT and 5'-ACCATTACGACTGATGTATAGTCT were used for the D9S171 marker. The oligonucleotide pairs used to detect the p16/CDKN2A gene were 5'-CTGGAGGAGGAGGACGAG (sense) and 5'-TCCCTTTTCGGAGAATCG (antisense) for exon 1, and 5'-AAAGGCTCTTTTCCGTATCCG (sense) and 5'-TGAGCTTTTGGAGTCTCAG (antisense) for exon 2.20) Homozygous deletions were detected by using comparative multiplex PCR assays.21, 22) Each PCR assay was carried out in a 20 μl reaction mixture containing 50 ng of genomic DNA, as described by Hamada et al.20) PCR products were analyzed by electrophoresis on a 3% agarose gel.

**Direct sequencing of PCR products** The K-ras gene mutation was analyzed by direct sequencing of PCR products using 50 ng of genomic DNA obtained from either A549, cloned cells, or the human lung adenocarcinoma cell line PC-14. Genomic DNA was amplified by PCR using the K-ras specific primer pairs, 5'-GACT-GAATATAAACTTGTGG (sense) and 5'-CTACATATTCG (antisense) with BigDye terminator cycle sequencing pre-mix kits (Amersham Pharmacia Bio-tech, Foster City, CA) and the ABI 310 DNA Sequence System (Perkin-Elmer, Tokyo).23)

**RESULTS**

**Selection of cell lines with high and low metastatic potential from A549 cells** Cells of the four cloned sub-populations were injected (5×10^5 cells/0.2 ml) into the tail vein of nude mice. Lung metastasis was graded from 0 to V (Table I). 2S and 11S showed high metastatic potential (Table I). Mice were killed when signs of illness were noted, and a single colony in the lung was removed, minced finely, and plated on tissue culture dishes in culture medium. Two high-metastatic cell lines, 2S Lu-4 (from 2S) and 11S Lu-1 (from 11S), were obtained. In the case of the low-metastatic cell lines, 8S and 16S, mice were killed at 141 days after injection of cells, and it was confirmed that there was no metastasis in the lung, lymph nodes and other organs (Table I).

**Experimental metastatic ability of the 4 cloned cell lines** Collected results from representative individual experiments to measure the metastatic capacity of the four cloned cell lines after injection of cells into the tail vein of the mice are presented in Table II. 2S Lu-4 and 11S Lu-1 had a higher lung colonization potential than the parental A549 cells. 2S Lu-4 and 11S Lu-1 caused metastases in all mice, with the median number of lung metastases per mouse being >300 and 289 respectively (Table II). Colonies produced by 2S Lu-4 became confluent on the surface of the lung (Fig. 1B). The original A549 produced 153 lung colonies (Table II). Mice given injections of 8S and 16S were sacrificed at 120 days (Table II) after the injection, but neither of these cell lines showed a marked ability to colonize the lungs or lymph nodes (Table II).

**Pathogenesis** We sought to quantify both the pathogenic and metastatic potential of the four cloned A549-derived cell lines compared to the parental A549 cells. We found significant differences in the survival of mice injected with the cloned cells compared to those injected with A549 cells (Fig. 2). Mice that had either 8S or 16S cell lines

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**Table I. Selection of Variant Cell Lines with Different Metastatic Potentials from Human Lung Adenocarcinoma A549**

| Metastatic selection cycle | Tumor cell line | Day of autopsy (range) | No. of mice with lung metastasis/total mice | Grade of metastasis<sup>a</sup> | Other visceral metastasis/total mice |
|---------------------------|-----------------|------------------------|--------------------------------------------|-------------------------------|-------------------------------------|
|                           |                 |                        |                                            |                               |                                      |
| 1                         | A549            | 86 (79→141)           | 2/4                                        | 0/4                           | 0/4                                 |
| 2S                        |                 | 68 (56→86)            | 5/5                                        | 0/5                           | 1/5 (LN)<sup>b</sup>               |
| 11S                       |                 | 90 (70→141)           | 5/5                                        | 1/5                           | 1/5 (LN)                            |
| 8S                        |                 | >141                   | 0/5                                        |                               |                                      |
| 16S                       |                 | >141                   | 0/5                                        |                               |                                      |

<sup>a</sup> Lung metastasis was graded from 0 to V. 0, tumor-free; I, histological evidence of tumor growth; II, <10 tumor foci; III, 10–100 tumor foci; IV, 100–200 tumor foci; V, >200 tumor foci.

<sup>b</sup> LN: lymph nodes.
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injected into the tail vein had in no deaths at 160 days post-injection. When mice given an injection of 16S were sacrificed at 160 days, only 1 to 5 mice showed a few lung colonies, which were less than 1 mm in diameter (Fig. 1E). The numbers of metastatic lung colonies in mice injected with A549 or the cloned cell lines (Table II) were shown to correlate with the survival times (Fig. 2).

**Morphology** Cells were plated and allowed to grow to 60% confluency. Cell morphology was examined by phase-contrast microscopy. Morphology was obviously

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**Table II. Experimental Lung and Lymph Node Metastases of A549-derived High- and Low-metastatic Human Lung Adenocarcinoma Cell Lines**

| Day of autopsy (range) | Cell line | No. of mice with lung metastasis/total mice | Median lung colonies/mouse (range) | No. of mice with lymph node metastasis/total mice |
|------------------------|-----------|--------------------------------------------|-----------------------------------|-----------------------------------------------|
| 79                     | A549      | 5/5                                        | 153 (57–261)                      | 0/5                                           |
| 79                     | 2S Lu-4   | 5/5                                        | >300                              | 0/5                                           |
| 79 (58–79)             | 11S Lu-1  | 5/5                                        | 289 (81–300)                      | 0/5                                           |
| 120                    | 8S        | 0/5                                        | 0                                 | 0/5                                           |
| 120                    | 16S       | 0/5                                        | 0                                 | 0/5                                           |

BALB/c nude mice were injected intravenously with \(5 \times 10^5\) viable cells and killed at 58–120 days after injection. The number of lung colonies in 2S Lu-4 and 11S Lu-1 were significantly different from that of parental A549 cells (Mann-Whitney U test, \(P<0.05\)).

Fig. 1. Experimental lung metastasis of A549, 2S Lu-4, 11S Lu-1, 8S and 16S. A, A549; B, 2S Lu-4; C, 11S Lu-1; D, 8S; E, 16S.
were small and flat-shaped, while the two low-metastatic cell lines (Fig. 3, D and E) were large and flat-shaped. None of the cell lines piled up and cells were not released from tissue culture dishes. The morphology of A549 (Fig. 3A) was heterogeneous.

**Growth and tumorigenicity** When A549 cells and the four cloned cells were inoculated subcutaneously into the mice (cell number of $1 \times 10^7$), A549 cells and the four cloned cells were highly tumorigenic. The growth of the primary tumors produced by the two high-metastatic cells was faster than that of A549 cells and the two low-metastatic cells (Fig. 4). Subcutaneous tumors formed by the inoculation of A549 and the four cloned cell lines did not produce lung or lymph node metastasis in any of the tumor-bearing mice.

**Colony morphology in collagen gels** The cloned cell lines and A549 were embedded in collagen gels. The morphology of colonies was observed at 14th days after seeding (Fig. 5) and the number of dispersed colonies was counted after 25 days in culture. The morphology of colonies was different between the high-metastatic and low-metastatic cell lines. The two high-metastatic cells formed.
round colonies. In these colonies, however, the outlines of individual cells could be seen, suggesting that their association is loose. Moreover, some cells were released from the main body of colonies and migrated out into the matrix (Fig. 5, B and C). Low-metastatic cell line 8S gave rise to colonies with round morphology, in which the cells were tightly associated with each other (Fig. 5D). Cells rarely migrated out of the colonies. Cell line 16S formed scattered colonies (Fig. 5E), but these scattered colonies died after 25 days (Table III). The two cell lines with high metastatic potential formed 29–44 clearly dispersed colonies per total of 50 colonies, and the two low-metastatic cell lines formed 4–22 weakly dispersed colonies (Table III). We observed the morphology of the colonies after 35 days in culture. Colonies formed by the low-metastatic cell lines had already died and cell debris was observed by phase-contrast microscopy, whereas colonies formed by the high-metastatic cell lines grew actively, and cells released from the colonies formed new colonies.

**Histology** Histological examination of tumors formed in nude mice inoculated in the back with 2S Lu-4 and 11S Lu-1 showed enhancement of angiogenesis (Fig. 6, A–C). Tumors formed by inoculation of 8S and 16S did not develop blood vessels (data not shown).
Expression of the VEGF, VEGF-B, VEGF-C and VEGF-D genes To examine whether expressions of the VEGF, VEGF-B, VEGF-C and VEGF-D genes are correlated with metastatic potential in A549 and the cloned cells, the cDNA fragments from these cells were subjected to PCR. The arrowheads show the 296 bp (VEGF), 357 bp (VEGF-B), 501 bp (VEGF-C) and 484 bp (VEGF-D) RT-PCR products (Fig. 7). These RT-PCR products were not different from each other.

Genetic analysis of the 9p21 region and the K-ras gene Genomic DNA obtained from A549 and each of the four cloned cell lines was subjected to PCR analysis of the homozygous deletions within the 9p21 region containing the p16 gene. All cell lines had an identical homozygous 9p21 deletion. However, deletions were not detected by PCR within the 9p21 DNA markers D9S1749 and D9S171 (Fig. 8). Finally, direct sequencing of PCR products showed that A549 and the four cloned cell lines had the same glycine-to-serine mutation (GGT→AGT) at amino acid position 12 in the K-ras gene (Fig. 9, B–D). The other two cloned cell lines had the same point mutation at amino acid position 12 (data not shown).

DISCUSSION

Metastasis is a major factor that contributes to the high death rate of people with lung carcinoma. Metastasis is a complex process that is dependent upon the fulfillment of a number of sequential steps. Many factors can influence the process of metastasis including detachment, motility, adhesion, invasion of cellular matrix, extravasation into the organ parenchyma, angiogenesis, and tumor growth. In the present study, we isolated two cell lines with high metastatic potential and two cell lines with low metastatic potential using the dilution plating technique from the human lung adenocarcinoma cell line A549. The high-

Table III. Dispersion of A549-derived High- and Low-metastatic Human Lung Adenocarcinoma Cell Lines in Collagen Gels

| Cell line | Number of dispersive colonies | Mean±SD |
|-----------|-------------------------------|---------|
| A549      | 7, 8, 7, 10                   | 8.00±1.22 |
| 2S Lu-4   | 40, 38, 44, 39                | 40.25±2.27b |
| 11S Lu-1  | 37, 30, 29, 32                | 32.00±3.08b |
| 8S        | 14, 18, 17, 22                | 17.75±2.86 |
| 16S       | 6, 4, 8, 8                    | 6.50±1.65 |
|           | (35, 35, 39, 32)a)            | (35.25±2.48) |

Cells were cultured in collagen gels. After 25 days in culture, the number of invasive colonies per total of 50 colonies was counted. For each cell line, four plates were used for the count and statistical analysis. Statistical significance of differences was calculated by using Student’s t test.

a) Colonies died after 25 days in culture.

b) Different from A549 at P<0.05.

Fig. 6. Histology of tumor tissues. A, A549; B, 2S Lu-4; C, 11S Lu-1. Arrowheads show blood vessels.
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metastatic cell lines produced many rapidly growing metastatic colonies on the lung. In the highly metastatic cell lines 2S Lu-4 and 11S Lu-1, a distinct phenotypic difference was observed and the in vivo growth rates of 2S Lu-4 and 11S Lu-1 were faster than those of 8S and 16S.

The four cloned cell lines were each cultured in collagen gels in order to assess their invasiveness. The high-metastatic cells formed round colonies that released cells from the main body and scattering of these cells resulted in the formation of three-dimensional colonies. It was previously reported that the scattering phenotype of highly metastatic cell lines was due to increase of migratory activity,18) and reduced levels of cell-adhesion molecules.25) We attempted to identify factors that correlated with cell scattering activity. Therefore, cells were seeded on coverslips coated with colloidal gold particles and the migration of the seeded cells was measured at 8 h and 15 h post-plating. We observed that the two low-metastatic cell lines migrated faster than the two high-metastatic cell lines (data not shown). Thus, the migratory activity of these cell lines did not correlate with their scattering activity. Colonies formed by the low-metastatic cell lines had already died, whereas colonies formed by the high-metastatic cell lines grew actively, and cells released from colonies formed new colonies after 35 days in culture. This

Fig. 7. Expression of the VEGF, VEGF-B, VEGF-C and VEGF-D genes in A549 and the cloned cell lines. Lane 1, minus template; lane 2, A549; lane 3, 2S Lu-4; lane 4, 11S Lu-1; lane 5, 8S; lane 6, 16S. The arrows indicate the 296 bp (A, VEGF), 357 bp (B, VEGF-B), 501 bp (C, VEGF-C), 484 bp (D, VEGF-D) RT-PCR products. DNA size markers of 603 bp and 310 bp are shown on the left. Human β-actin transcripts (E, 500 bp) were used to standardize the amount of RNA.

Fig. 8. PCR analysis of chromosome band 9p21 markers in A549 and the cloned cell lines. Lane 1, N417; lane 2, A549; lane 3, 2S Lu-4; lane 4, 11S Lu-1; lane 5, 8S; lane 6, 16S. 9p markers are arranged from telomeric (D9S1749) to centromeric (D9S171). N417 does not delete p16 gene.

Fig. 9. Mutation of the K-ras gene in A549 and the cloned cell lines. A, PC-14; B, A549; C, 2S Lu-4; D, 8S. The mutation (GGT → AGT) at codon 12 was detected (reverse sequence) and the mutated nucleotides are underlined. The K-ras gene of PC-14 is wild-type.
suggests that the activities of the growth factors were increased in the high-metastatic cells.

It has been demonstrated that an adequate blood supply is critical for tumor development and progression.26–29 The initiation of angiogenesis within a metastatic tumor depends upon a critical balance between angiogenic activators and inhibitors that are up- or down-regulated at specific stages of tumor development. We observed that the tumors formed by inoculation of highly metastatic cells into the subcutis of nude mice developed blood vessels. The expression patterns of the VEGF family genes in relation to the metastatic potential were analyzed by RT-PCR. The VEGF family genes, VEGF, VEGF-B, VEGF-C and VEGF-D, were similarly expressed in A549 and the cloned cells. These results suggest that the expressions of the VEGF family genes promote tumor angiogenesis and growth,30–34 but do not contribute to the first step of metastasis. The metastatic potential of A549 may be governed by, at least, an enhanced growth rate, and greater invasiveness and angiogenesis.

A large number of genes must be involved in the metastasis of A549 cells. The four cloned cell lines exhibited the same homozygous deletions in the 9p21 region containing the p16 gene,35–39 and the same glycine-to-serine mutation (GGT → AGT) at amino acid position 12 in the K-ras gene of parental A549 cells.40 These results suggest that the four cloned cell lines that originated from a heterogeneous population of A549 cells have unique characteristics. Thus, deletion within p16 and mutation of the K-ras gene do not fully explain the metastatic ability of the two highly metastatic cell lines, suggesting that these cell lines may offer a means to identify novel factors that contribute to the mechanism of metastasis of human lung cancer. We have already established and characterized high-metastatic cells, Lu-2, Lu-7, Lu-4, Lu-1 and Lu-5, and low-metastatic cells, 7S, 3S, 8S and 13S from PC-14 human lung adenocarcinoma.9 We have already used mRNA differential display methods to detect genes associated with the metastatic potential of three high-metastatic cell lines and three low-metastatic cell lines derived from PC-14. We isolated a novel gene, the Arp11 gene,10 that was identified as being expressed in low-metastatic cells but not in high-metastatic cells and which belongs to the actin-related family. The Arp11 showed 89% amino acid identity with human Arp3, which is one of the actin-related proteins. The Arp3 constructs the Arp2/3 protein complex, which promotes actin polymerization.41, 42 Other actin-related proteins correlate to the regulation of growth and transcription.43, 44 We have transfected Lu-2 cells derived from PC-14 with pcDNA3-Arp11 using “LipofectAMINE 2000” reagent, and an analysis of the roles of this gene and other genes specifically expressed in low- or high-metastatic cell lines from PC-14 and A549 is in progress.

ACKNOWLEDGMENTS

We thank Dr. J. D. Minna for the gift of A549 human lung adenocarcinoma. We thank Dr. H. Tsuda for the histological experiments and Dr. J. Yokota for the experiments on genetic analysis of the 9p21 region and the K-ras gene.

(Received June 21, 2001/Revised September 3, 2001/Accepted October 6, 2001)

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