Biochemical and Biophysical Research Communications **280,** 61–67 (2001)
doi:10.1006/bbrc.2000.4059, available online at http://www.idealibrary.com on IDEAL®

Isolation of a Novel Actin-Related Gene Expressed in Low-Metastatic PC-14 Human Lung Adenocarcinoma

Nobuko Shindo-Okada[1] and Kimihiro Shimizu
Biology Division, National Cancer Center Research Institute, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

Received November 22, 2000

In this article, we report the identification of differentially expressed genes associated with the metastatic potential of PC-14 human lung adenocarcinoma. The mRNA differential display method was applied to compare mRNAs from six cloned PC-14 cell lines representing different metastatic potentials. A novel gene was identified as being expressed in low-metastatic cells but not in high-metastatic cells. Sequence analysis revealed that this novel gene has an open reading frame of 210 amino acid residues showing 89% amino acid identity with human Arp3, which is one of the actin-related proteins. This gene was designated the Arp11 gene (the 11th Actin-related gene). The Arp11 gene was mapped to human chromosome 7q32–36. Southern blot and PCR analyses show that all of the high-metastatic variant cells have not rearrangements and deletions of Arp11 allele. Expression of the Arp11 gene may regulate the metastatic potential of PC-14 human lung adenocarcinoma.

Key Words: **lung cancer; human lung adenocarcinoma; PC-14 cells; mRNA differential display; actin-related gene; Arp11; metastasis; established cell lines; high-metastatic cells; low-metastatic cells.**

A simple and effective method for screening a large number of cell lines is the recently developed mRNA differential display which can identify genes that are differentially expressed in cells (1–3). We had previously used this technique to detect genes associated with the metastatic potential of three high-metastatic (4, 5) and three low- or non-metastatic clones (6) of K-1735 mouse melanoma. We detected 8 genes, β-toropomyosin, inhibin/actibin βB subunit, macrophage colony-stimulating factor (M-CSF), and 2 unknown genes, Elm-1 (expressed in low-metastatic cell) (7, 8) and Elm-2 which were specifically expressed in the low-metastatic cell lines from K1735, and integrin α6 and 2 unknown genes, and Ehm-1 (expressed in high-metastatic cells) (Sox21) (9) and Ehm-2 which were expressed in the high-metastatic cell lines from K-1735 (7). The deduced Elm-1 belongs to the CCN (connective tissue growth factor [CTGF], Cyr61/Cef10, neuroblastoma overexpressed gene [Nov]) family protein, which consist of secreted cystein-rich proteins with growth regulatory functions (10–13). Transfectants which highly expressed Elm-1 did not produce lung metastasis by injection into the tail vein of mice. This suggests that multiple genes interact with the induction of lung colonies by melanoma (8).

In this paper, to identify genes that relate to lung cancer metastasis, we used three established high-metastatic clones, Lu-2, Lu-7 andLu-4 and three low-metastatic clones, 3S, 7S, and 13S (unpublished results, Shindo-Okada, N., *et al.*) for mRNA differential display. We already identified 27 candidates differentially expressed between the three low-metastatic cells and the three high-metastatic cells using a 192 primer set. Among them, a novel gene, the Arp11 gene, was identified as being expressed in low-metastatic cells but not in high-metastatic cells. The Arp11 gene encodes a predicted amino acid sequence showing 89% amino acid identity with Arp3 protein, which is one of Arp2/3 complex (14, 15). The human Arp2/3 complex contains Arp2 and Arp3, and five others, referred to as p41-Arc, p34-Arc, p21-Arc, p20-Arc and p16-Arc (Arp complex) (16). These seven subunits and the other actin-related proteins have been maintained during evolution and interact with actin polymerization and the regulation of growth and transcription (17). These suggest the possibility that the Arp11 gene may function a role in the suppression of metastatic potential in PC-14 human lung adenocarcinoma.

MATERIALS AND METHODS

Tumor lines. PC-14 human lung adenocarcinoma cells, derived from a previously untreated patient with pulmonary adenocarcinoma was kindly provided by Prof. Y. Hayato, Tokyo Medical College. The five high-metastatic cell lines, Lu-2, Lu-7, Lu-4, Lu-1, and

Sequence data from this paper has been deposited with the DDBJ/GenBank/EMBL data libraries under Accession No. AB039791.

[1] To whom correspondence and reprint requests should be addressed. Fax: 81-3-3542-0807. E-mail: nokada@gan2.res.ncc.go.jp.

0006-291X/01 $35.00

Lu-5 and the four low-metastatic cell lines, 3S, 7S, 8S, and 13S which were established from PC-14, was used (unpublished results, Shindo-Okada, N., *et al.*).

Conditions of culture. PC-14 and its variant cells with different metastatic potential were grown in RPMI 1640 medium (Nissei Pharmaceutical Corp.) supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml) in a highly humidified atmosphere of 5% CO_2 at 37°C. The cell lines were subcultured once or twice a week as necessitated by growth rate.

Differential display of mRNA. Six PC-14 clones, Lu-2, Lu-7, Lu-4, 3S, 7S, and 13S, representing different metastatic potentials were used as sources of RNA. Cells at 60–80% confluency were harvested and subjected to RNA isolation using a Fast Track mRNA Isolation kit (Invitrogen) according to the manufacturer's recommendations. Differential display was performed using a RNA map kit (Gene Hunter Corp.) according to the manufacturer's recommendation with slight modifications. One tenth μg of poly(A)$^+$ RNA was reverse transcribed with T_{12}MA (where M may be dA, dG, or dC) as a primer, followed by PCR amplification in the presence of [^{32}P]dCTP (Amersham) using T_{12}MA (antisense strand) and 5′-GGAATTC-TGGATTCCATCC-3′ (sense strand) according to the standard method (7, 9).

Cloning and sequencing of cDNA. The cDNA fragment which was expressed only in low-metastatic PC-14 cells was reamplified by PCR and cloned into the pCR II vector using the TA Cloning System (Invitrogen) according to the manufacturer's instruction. The insert was sequenced using the ABI 373S DNA Sequencing System (Perkin Elmer) with a II dye terminator cycle sequencing kit (Amersham). The novelty of the isolated clone was determined by computer search and comparison against the DDBJ/GenBank/EMBL DNA databases (18).

Screening of cDNA library. 6.5×10^5 recombinant phage clones of cDNA library from low-metastatic PC-14 cell line, 7S constructed in lambda ZAPII using cDNA Synthesis System Plus (Amersham) was screened with cloned cDNA fragment that was isolated by mRNA differential display to isolate full-length cDNA clones (19). Isolated clones were sequenced by ABI 373S DNA Sequencing System with a II dye terminator cycle sequencing kit. DNA sequences were aligned, examined for open reading frames, and compared with DNA sequences in the DDBJ/GenBank/EMBL databases and with amino acid sequences in the Swiss-Prot and PIR protein databases using the FASTA and BLAST programs.

Northern and Southern blot analyses. Five micrograms of poly(A)$^+$ RNA isolated from PC-14 and the 9 cell lines was size fractionated on 1.0% denaturing formaldehyde agarose gel for Northern blot and 10 μg of *Eco*R1-digested genomic DNA was fractionated on 0.8% agarose gel for Southern blot and transferred onto a Hybond-N+ membranes (Amersham). Northern and Southern blot hybridization was performed at 42°C for 40 h under stringent conditions and the membranes were washed with 0.1× SSC and 0.1% SDS at 56°C, as previously described (20). To confirm the amounts of mRNA loaded in each lane, the blots were hybridized afterwards with a human β-actin probe. To confirm the amounts of *Eco*R1-digested genomic DNA loaded in each lane, 0.8% agarose gel was stained with ethidium bromide. Northern and Southern blot filters hybridized with cloned cDNA fragment were exposed for 40 days and 60 days, respectively. Northern blot filter hybridized with human β-actin probe was exposed for 20 h.

PCR and RT-PCR analyses. One hundred nanograms of genomic DNA was amplified by the primer sets, 5′-GAACCTCCACTCAAT-3′ (sense strand) and 5′-TGCCTGAACTGCAATGT-3′ (antisense strand), 5′-TCAACAGCTGCTAAGGG-3′ (sense strand) and 5′-CTTCTTCTGGTTGATCGC-3′ (antisense strand), and 5′-CTATC-CTCAAGGTCACG-3′ (sense strand) and 5′-ATCTCAAATAAA-AGGCTACG-3′ (antisense strand) and corresponding to nucleotides 143–157, 222–238, 414–430, 584–601, 775–791, and 834–853, respectively. PCR products were fractionated on 3% agarose gel and stained with ethidium bromide. One hundred nanograms of poly(A)$^+$ RNA isolated from human kidney, stomach, small intestine, spleen, bone marrow, brain, uterus, testis, thymus, placenta, skeletal muscle, mammary gland, lung, fetal liver, and fetal kidney was reverse transcribed with oligo dT_{12} as a primer, subjected to PCR using 5′-CCACAGAACCTCCACTCAAT-3′ (sense strand) and 5′-ATCTCAAATAAAAGGCTACG-3′ (antisense strand) corresponding to nucleotides 222–238 and 834–853, respectively and fractionated on 0.7% agarose gel and stained with ethidium bromide.

RESULTS AND DISCUSSION

Isolation of genes expressed in low-metastatic cells. To identify genes differentially expressed in association with the metastatic potential of PC-14 cells, poly(A)$^+$ RNAs from 3 high-metastatic cell lines, Lu-2, Lu-7 and Lu-4, and 3 low-metastatic cell lines, 3S, 7S and 13S were analyzed by the mRNA differential display method. By using a set of primers (see Materials and Methods), cDNA fragment (fragment 1) was amplified in low-metastatic cells and not in high-metastatic cells (Fig. 1A). Then, the fragment was cloned into the TA cloning vector, and several clones were subjected to DNA sequencing. Fragment 1 consisted of 454 nucleotides, and the sequence was not identical to and not significantly homologous to any recorded sequences in the DDBJ/GenBank/EMBL DNA databases. Therefore, we screened 6.5×10^5 recombinant phage clones of the cDNA library from low-metastatic 7S cells to isolate a full-length cDNA clone. Three candidate clones were obtained, sequenced, and aligned. We assembled a composite 1218-bp transcript except poly A tail using the sequences of clones obtained from the cDNA library. We verified that the assembled sequence was derived from a single gene by reverse transcription-PCR. The sequence of fragment 1 corresponded to the 3′ region of this gene.

Northern blot analysis. The expression pattern in correlation with metastatic potential was then confirmed by Northern blot analysis. The novel gene was expressed in the 4 low-metastatic cells and parental PC-14 cells but not in the 5 high-metastatic cells (Fig. 1B). The expression of the actin-related protein family gene was usually one-hundredths in comparison with that of actin, but the expression of the Arp11 gene showed under one-hundredths (Fig. 1B).

Homology of a novel gene with the Arp3 gene. The novel gene showed the highest homology to the human Arp3 gene. The novel gene cDNA encodes a predicted protein of 210 amino acids using the first 5′ methionine, which is located 123 bp downstream of in-frame stop codon (Fig. 2A) and is 75% identical to the human Arp3 cDNA (data not shown). The amino acid sequence from codon 1 (Met) to codon 188 (Lys) of this protein shows 89% identity with the amino acid sequence from codon 130 (Met) to codon 317 (Lys) of Arp3 protein (Fig. 2B). Arp3 is one of actin-related proteins. Arp3 constructs the Arp2/3 protein complex, consisting of seven

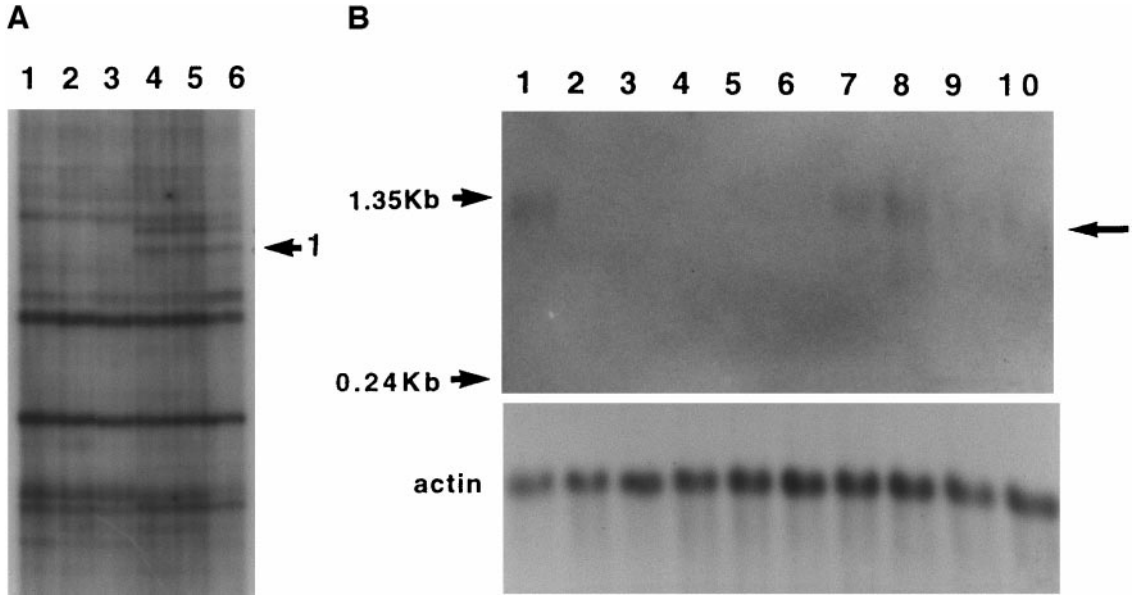

FIG. 1. Differential expression of the Arp11 gene in association with metastatic potential of PC-14. (A) Detection of expression of the Arp11 gene by mRNA differential display using a 3′ primer $T_{12}M$(dA, dG, dc mix)A and a 5′ primer GGAATTCTGGATTCCATCC. Lane 1, Lu-2; lane 2, Lu-7; lane 3, Lu-4; lane 4, 3S; lane 5, 7S; lane 6, 13S. Arrowhead indicates the position of the Arp11 cDNA fragment1 (454 bp). (B) Northern blot analysis of the Arp11 gene. Five micrograms per lane of poly(A)$^+$. RNA from parental PC-14 (lane 1), high-metastatic cells (lane 2, Lu-2; lane 3, Lu-7; lane 4, Lu-4; lane 5, Lu-1; lane 6, Lu-5), and low-metastatic cells (lane 7, 3S; lane 8, 7S; lane 9, 13S; lane 10, 8S) were subjected to Northern blot analysis. Arrows indicate the position of 1.2 Kb of the Arp11 transcript. Human β-actin transcripts standardize the amount of RNA on the Northern blot membrane. RNA size markers of 1.35 Kb and 0.24 Kb are shown on the left.

subunits which include the actin-related proteins Arp 2 and Arp 3, and five others referred to as p41-Arc, p34-Arc, p21-Arc, p20-Arc, and p16-Arc and these seven proteins were purified by affinity chromatography on profilin-agarose. The Arp complexis highly conserved in various species including human (21) and expressed in diverse organs. The Arp2/3 protein complex promotes actin polymerization at the surface of Listeria monocytogenes and interacts with cell shape change and migration (15, 16). It was previously reported that the organization of actin-containing microfilament bundles was different between low- and high-metastatic K-1735 cells (22). Nearly all the low-metastatic cells exhibited prominent actin bundles while nearly all the high-metastatic cells had poor actin organization. The Arp3 gene was assigned to be distal between the D2S121 and the D2S110 loci at 2q13–14.1, based on the mapping data in GeneMap'98 (http://www.ncbi.nlm.nih.gov/genemap98). Arp1 is a component of a multi-protein assembly that promotes dynein-based vesicle motility (23, 24) and may be associated with centrosomes (25). Arp1 binds nucleotide, cocycles with polymerized actin (26), and forms a short filament resembling filamentous actin in the dynatin complex (27). Human Arp1, Arp2, Arp3 and a novel protein are, respectively, 53.8%, 47.7%, 36.9% and 33.3% identical to human skeletal muscle α-actin (DDBJ/GenBank/EMBL accession number; P02568). Predicted amino acid sequence of a novel gene is compared with those of human skeletal muscle α-actin, Arp1, Arp2 and Arp3. Residues identical between a novel gene and one or several of the sequences analyzed are boxed (Fig. 2C). The possible conservation of ATP binding motifs (14) was found in the Arp11 gene. Letters (n) below the alignment marks the nucleotide binding (Fig. 2C). Arp4 (13E) and Arp5 (53D) (DDBJ/GenBank/EMBL accession number; Arp4, L25314; Arp5, X78487) were identified in *Drosophila* (28, 29) and a partial fragment of human Arp5 was isolated (DDBJ/GenBank/EMBL accession number; AA902650). Computer searches for actin-related proteins reveal a family of ten proteins in yeast *Saccharomyces cerevisiae* (Arp1–Arp10) (30). Arp7 and Arp9 are shared functional components of the chromatin-remodeling complexes RSC and SWI/SNF (17, 31) and function in a very important role in transcriptional regulation. In human, SWI/SNF and BAF, which is an actin-related protein, interact with similar functions to those of yeast (32). A novel protein was different from these actin-related proteins. Therefore, this gene was designated the Arp11 gene (the 11th actin-related gene). The Arp11 gene was assigned to be distal between the D7S2450 and the D7S550 loci at 7q32–36, based on the mapping data in GeneMap'98 (http://www.ncbi.nlm.nih.gov/genemap98). Arp11 may have an important role in biological activity and is likely to have properties and functions different from those of other actin-related proteins.

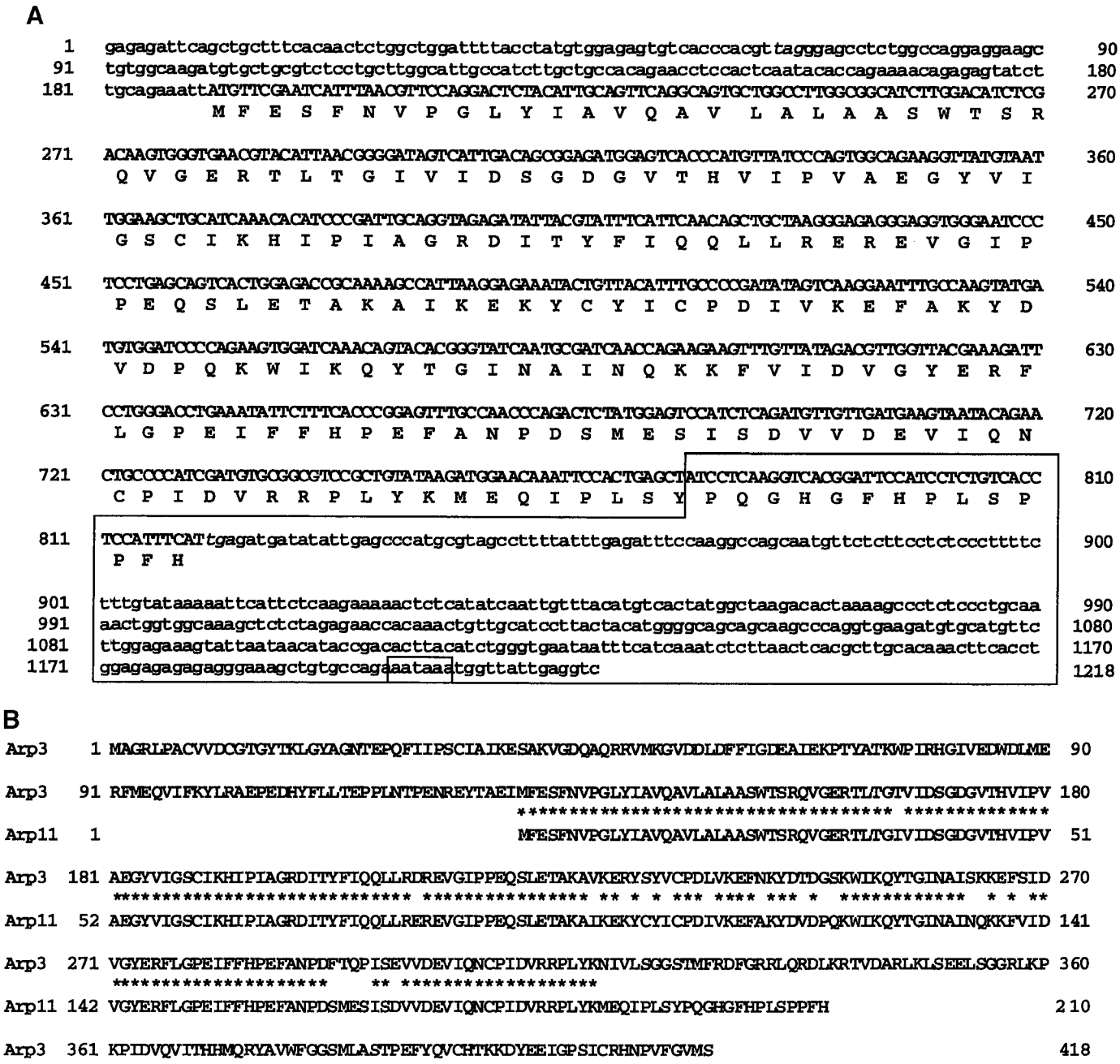

FIG. 2. Nucleotide and predicted amino acid sequence of the Arp11 gene. (A) The sequences of fragment 1 are shown in the large box under the corresponding sequences of the isolated gene. One hundred and ten Amino acids are shown in their one letter form under the corresponding nucleotide sequence. An in-frame 5′ stop codon and the predicted termination stop codon are indicated in italics. A potential polyadenylation signal is shown in the small box. (B) Amino acid sequences of the Arp11 gene and the Arp3 gene. Amino acid sequences of Arp3 are available from DDBJ/GenBank/EMBL DNA databases under Accession No. p32391. (C) Alignment and homology of amino acid sequences of the Arp11 gene, the α-actin gene, the Arp1 gene, the Arp2 gene and the Arp3 gene. Predicted amino acid sequence of Arp11 is compared with those of human skeletal muscle α-actin, Arp1, Arp2 and Arp3. The optimal alignment of the sequences was found by inserting the minimum number of gaps to maximize the number of matches. Residues identical between Arp11 and one or several of the sequences analyzed are boxed. Letters (n) below the alignment mark the nucleotide binding.

Expression of the Arp11 gene in multiple tissues. To compare the expression of the Arp11 gene in human kidney, stomach, small intestine, spleen, bone marrow, brain, uterus, testis, thymus, placenta, skeletal muscle, mammary gland, lung, fetal liver, and fetal kidney. cDNA fragments from these tissues were subjected to PCR using 5′-CCACAGAACCTCCACTCAAT-3′ (sense strand) and 5′-ATCTCAAATAAAAGGCTACG-3′ (anti-

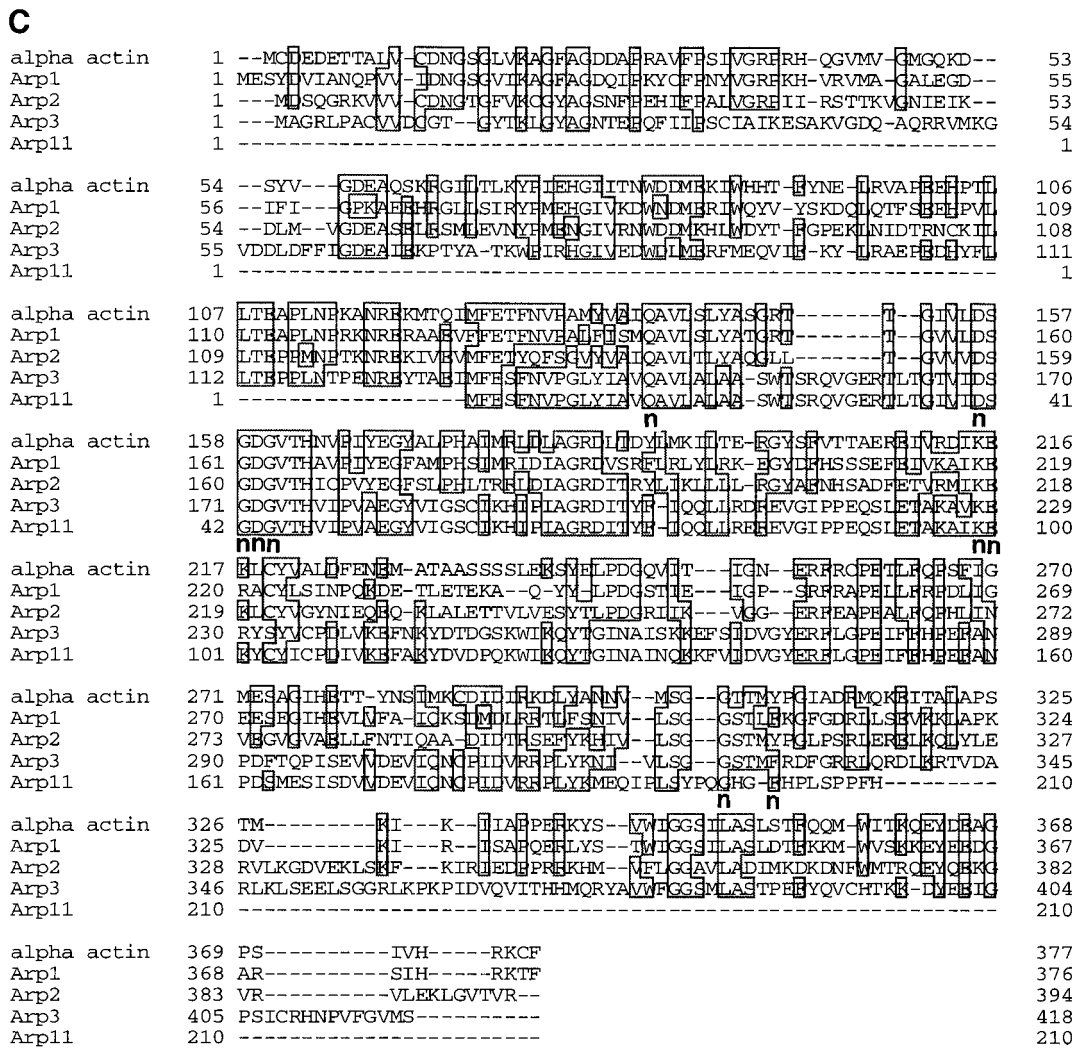

FIG. 2—*Continued*

sense strand). The arrowhead shows a 723 bp RT-PCR product (Fig. 3). The Arp11 gene was expressed in human kidney, stomach, spleen, bone marrow, uterus, testis, placenta, skeletal muscle, mammary gland, lung, fetal liver, and fetal kidney, but was not detected in small intestine, brain, and thymus (Fig. 3).

Southern blot and PCR analyses. Southern signals were detected in *Eco*R1-digested genomic DNA from PC-14 and the 9 cloned cells (Fig. 4A) and two fragments were detected by Southern blot using a DNA probe for the Arp11 gene corresponding to nucleotides 143–853 of the Arp11 cDNA fragment (data not shown). These fragments were not different from each other. Fragment 1 (143–238 nucleotides [nts]) consisting of 48 nts of the 5′-untranslated region and 48 nts of the coding region, fragment 2 (414–601 nts) consisting

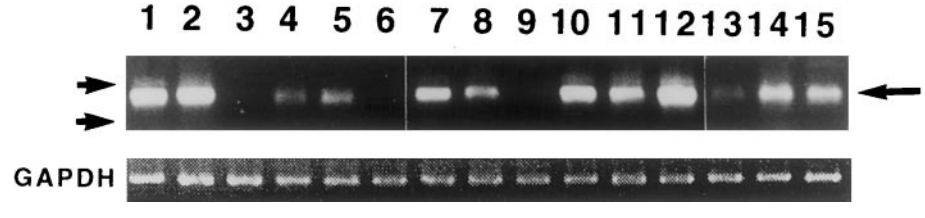

FIG. 3. Expression of the Arp11 gene in multiple tissues. The arrow shows the 723-bp RT-PC product. Lane 1, human kidney; lane 2, stomach; lane 3, small intestine; lane 4, spleen; lane 5, bone marrow; lane 6, brain; lane 7, uterus; lane 8, testis; lane 9, thymus; lane 10, placenta; lane 11, skeletal muscle; lane 12, mammary gland; lane 13, lung; lane 14, fetal kidney; and lane 15, fetal liver. DNA size markers of 872 bp is shown on the left. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts (491 bp) standardize the amount of RNA.

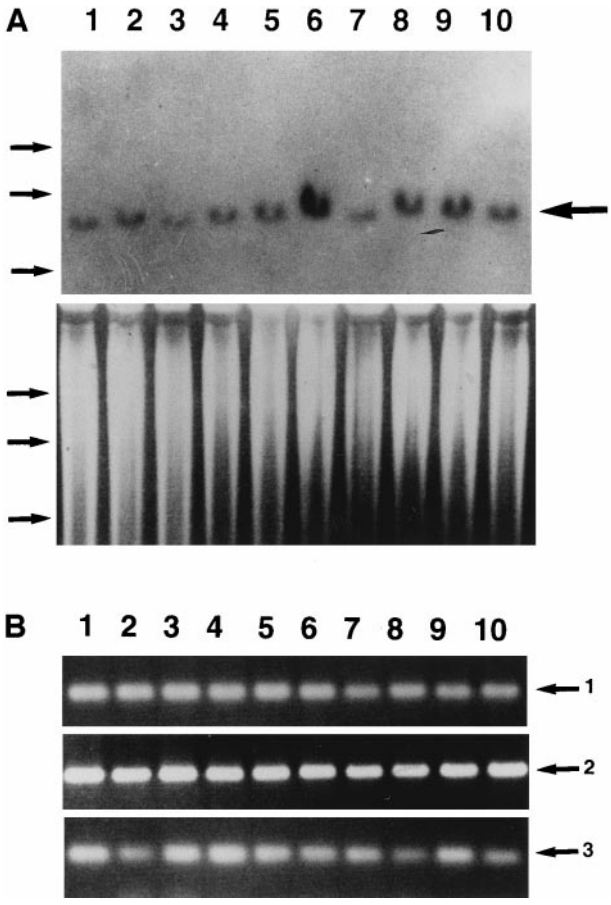

FIG. 4. Southern blot and PCR analyses of the Arp11 gene. (A) Southern blot analysis of the Arp11 gene. Ten micrograms per lane of genomic DNA from parental PC-14 (lane 1), high-metastatic cells (lane 2, Lu-2; lane 3, Lu-7; lane 4, Lu-4; lane 5, Lu-1; lane 6, Lu-5), and low-metastatic cells (lane 7, 3S; lane 8, 7S; lane 9, 13S; lane 10, 8S) were subjected to Southern blot analysis. The agarose gel was stained with ethidium bromide to confirm the amount of DNA on the Southern blot membrane. The right arrowhead shows Southern signals in *Eco*R1-digested genomic DNA from PC-14 and the 9 cloned cells. DNA size markers of 6.56, 4.36, and 2.37 Kb are shown on the left. (B) PCR analysis of the Arp11 gene. Lane 1, PC-14; lane 2, Lu-2; lane 3, Lu-7; lane 4, Lu-4; lane 5, Lu-1; lane 6, Lu-5; lane 7, 3S; lane 8, 7S; lane 9, 13S; and lane 10, 8S. Arrows 1, 2, and 3 indicate the 96 nucleotides (5′-untranslated region and coding region), 188 nucleotides (coding region) and 79 nucleotides (5′-coding region and untranslated region) of PCR products of the Arp11 gene.

of 188 nts of the coding region and fragment 3 (775–853 nts) consisting of 46 nts of the coding region and 33 nts of the 3′-untranslated region of PCR products from genomic DNA were detected in all the cell lines (Fig. 4B). These showed that all of the 5 high metastatic cell lines have not rearrangements and deletions of Arp11 allele. These suggest the possibility that metastasis is induced by not only rearrangement and deletion of the tumor suppressor genes but expressional regulation of the genes interacting with metastatic potential. Now, Lu-2 cells were transfected with pcDNA3-Arp11 using LipofectAMINE 2000 reagent. Cell clones resistant to geneticine (G418) are assayed. The characterization of the Arp11 gene should allow us a critical analysis of the molecular and biological mechanisms of metastasis in PC-14 cells.

ACKNOWLEDGMENT

We thank Prof. Y. Hayato for the gift of PC-14 human lung adenocarcinoma.

REFERENCES

1. Liang, P., and Pardee, A. B. (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* **257,** 967–971.
2. Liang, P., Averboukh, L., and Pardee, A. B. (1993) Distribution and cloning of eukaryotic mRNAs by means of differential display: Refinements and optimization. *Nucleic Acids Res.* **21,** 3269–3276.
3. Bauer, D., Muller, H., Reich, J., Reidel, H., Ahrenkiel, V., Warthoe, P., and Strauss, M. (1993) Identification of differentially expressed mRNA species by an improved display technique (DDRT-PCR). *Nucleic Acids Res.* **21,** 4272–4280.
4. Talmadge, J. E., Wolman, S. R., and Fidler, I. J. (1982) Evidence for the clonal origin of spontaneous metastases. *Science* **217,** 361–363.
5. Fidler, I. J., and Talmadge, J. E. (1986) Evidence that intravenously derived murine pulmonary melanoma metastases can originate from the expansion of a single tumor cell. *Cancer Res.* **46,** 5167–5171.
6. Aukerman, S. L., Price, J. E., and Fidler, I. J. (1986) Different deficiencies in the prevention of tumorigenic low metastatic murine K-1735b melanoma cells from producing metastases. *J. Natl. Cancer Inst.* **77,** 915–924.
7. Hashimoto, Y., Shindo-Okada, N., Tani, M., Takeuchi, K., Toma, H., and Yokota, J. (1996) Identification of genes differentially expressed in association with metastatic potential of K-1735 murine melanoma by messenger RNA. *Cancer Res.* **56,** 5266–5271.
8. Hashimoto, Y., Shindo-Okada, N., Tani, M., Nagamachi, Y., Takeuchi, K., Shiroishi, T., Toma, H., and Yokota, J. (1998) Expression of the *ELM1* gene, a novel gene of the CCN (connective tissue growth factor, Cyr61/Cef10, and neuroblastoma overexpressed gene) family, suppresses in vivo tumor growth and metastasis of K-1735 murine melanoma cells. *J. Exp. Med.* **187,** 289–296.
9. Tani, M., Shindo-Okada, N., Hashimoto, Y., Shiroishi, T., Takenoshita, S., Nagamachi, Y., and Yokota, J. (1997) Isolation of a novel *Sry*-related gene that is expressed in high-metastatic K-1735 murine melanoma cells. *Genomics* **39,** 30–37.
10. Bradham, D. M., Igarashi, A., Potter, R. L., and Grotendorst, G. R. (1991) Connective tissue growth factor: A cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product CEF-10. *J. Cell Biol.* **114,** 1285–1294.
11. O'brien, T. P., Yang, G. P., Sanders, L., and Lau, L. (1990) Expression of cyr61, a growth factor-inducible immediate-early gene. *Mol. Cell. Biol.* **10,** 3569–3577.
12. Kireeva, M. I., Mo, F-E., Yang, G., and Lau, L. F. (1996) Cyr61, a product of a growth factor-inducible immediate-early gene, promotes cell proliferation, migration, and adhesion. *Mol. Cell. Biol.* **16,** 1326–1334.
13. Martinerie, C., Huff, V., Joubert, I., Badzioch, M., Saunders, G., Strong, L., and Perbal, B. (1994) Structural analysis of the

human *nov* proto-oncogene and expression in Wilms tumors. *Oncogene* **9,** 2729–2732.

14. Kelleher, J. F., Atkinson, S., and Pollard, T. (1995) Sequences, structural models, and cellular localization of the actin-related proteins Arp2 and Arp3 from Acanthamoeba. *J. Cell Biol.* **131,** 385–397.
15. Welch, M. D., Iwamatsu, A., and Mitchison, T. J. (1997) Actin polymerization is induced by Arp2/3 protein complex at the surface of Listeria monocytogenes. *Nature* **385,** 265–269.
16. Welch, M. D., DePace, A. H., Verma, S., Iwamatsu, A., and Mitchison, T. J. (1997) The human Arp2/3 complex is composed of evolutionally conserved subunits and is localized to cellular regions of dynamic actin filament assembly. *J. Cell Biol.* **138,** 375–384.
17. Peterson, C. L., Zhao, Y., and Chait, B. T. (1998) Subunits of the yeast SWI/SNF complex are members of the actin-related protein (ARP) family. *J. Biol. Chem.* **273,** 23641–23644.
18. Pearson, W. R., and Lipman, D. J. (1988) Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci. USA* **85,** 2444–2448.
19. Tanaka, M., Sasaki, H., Kino, I., Sugimura, T., and Terada, M. (1992) Genes preferentially expressed in embryo stomach are predominantly expressed in gastric cancer. *Cancer Res.* **52,** 3372–3377.
20. Yokota, J., Akiyama, T., Fung, Y-K., Benedict, W. F., Hanaoka, M., Wada, M., Terasaki, T., Shimosato, Y., Sugimura, T., and Terada, M. (1988) Altered expression of the retinoblastoma (*RB*) gene in small-cell carcinoma of lung. *Oncogene* **3,** 471–475.
21. Frankel, S., and Mooseker, M. S. (1988) The actin-related proteins. *Curr. Opin. Cell Biol.* **8,** 30–37.
22. Volk, T., Geiger, B., and Raz, A. (1984) Motility and adhesive properties of high- and low-metastatic murine neoplastic cells. *Cancer Res.* **44,** 811–824.
23. Lees-Miller, J. P., Helfman, D. M., and Schroer, T. A. (1992) A vertebrate actin-related protein is a component of a multisubunit complex involved in microtubule-based vesicle motility. *Nature* **359,** 244–246.
24. Paschal, B. M., Holzbaur, E. L. F., Pfister, K. K., Clark, S., Meyer, D. I., and Valle, R. B. (1993) Characterization of a 50-kDa polypeptide in cytoplasmic dynein preparations reveals a complex with $p150^{GLUED}$ and a novel actin. *J. Biol. Chem.* **268,** 15318–15323.
25. Clark, S. W., and Meyer, D. I. (1992) Centractin is an actin associated with the centrosome. *Nature* **359,** 246–250.
26. Melki, R., Vainberg, I. E., Chow, R. L., and Cowan, N. J. (1993) Chaperonin-mediated folding of vertebrate actin-related protein and γ-tubulin. *J. Cell Biol.* **122,** 1301–1310.
27. Schafer, D. A., Gill, S. R., Cooper, J. A., Heuser, J. E., and Schroer, T. A. (1994) Ultrastructural analysis of the dynacin complex an actin-related protein is a component of a filament that resembles actin. *J. Cell Biol.* **126,** 403–412.
28. Frankel, S., Heintzelman, M. B., Artravanis-Tsakonas, S., and Moosaker, M. S. (1994) Identification of a divergent actin-related protein in Drosophila. *J. Mol. Biol.* **235,** 1351–1356.
29. Fyrberg, C., Ryan, L., Kenton, M., and Fyrberg, E. (1994) Genes encoding actin-related proteins of Drosophila. *J. Mol. Biol.* **241,** 498–503.
30. Poch, O., and Winsor, B. (1997) Who's who among Saccharomyces cerevisiae actin-related proteins? A classification and nomenclature proposal for a large family. *Yeast* **13,** 1053–1058.
31. Cains, B. R., Erdjument-Bromage, H., Temps, P., Winston, F., and Kornberg, R. D. (1998) Two actin-related proteins are shared functional components of the chromatin-remodeling complexes RSC and SWI/SNF. *Mol. Cell.* **2,** 639–651.
32. Zhao, K., Wang, W., Rando, O. J., Xue, Y., Swiderek, K., Kuo, A., and Crabtree, G. R. (1998) Rapid and phosphoinositol-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. *Cell* **25,** 625–636.