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Gelsolin: A Candidate for Suppressor of Human Bladder Cancer

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Abstract

Human transitional cell carcinomas of the bladder frequently reveal chromosomal abnormalities that span a range between chromosome 9pl2 and 9qter, even at early stages of bladder carcinogenesis. Because the gene that encodes an actin-regulatory protein, gelsolin, is localized in chromosome 9q33, we examined the expression of gelsolin in a number of human bladder cancer cell lines and tissues. In all 6 cell lines and in 14 of the 18 tumor tissues (77.8%), gelsolin expression was undetectable or extremely low in comparison with its expression in normal bladder epithelial cells. Furthemore, upon the introduction of the exogenous human or mouse authentic gelsolin cDNA into a human bladder cancer cell line, UMUC-2, gelsolin transfectants of UMUC-2 greatly reduced the colony-forming ability and the tumorigenicity in vivo. These results suggest that gelsolin plays a key role as a tumor suppressor in human urinary bladder carcinogenesis.

Introduction

Bladder cancers, which generally originate in urinary transitional cells, exhibit a diversity of biological features. Superficial papillary TCCs[4] of the bladder show multicentricity and a high rate of tumor recurrence, although most patients with superficial tumors tend to have low-grade and low-stage tumors. Carcinomas in situ, flat superficial lesions, show initially a high propensity to become invasive tumors (1, 2). Earlier cytogenetic studies have revealed various chromosomal anomalies in TCCs. The

rate of loss of heterozygosity in chromosome 9q has been found to be approximately 70% in superficial papillary tumors (1). Advanced tumors have displayed a mixture of aberrant 3p, 5q, 9p, 9q, and 17p (1-5). The alteration of chromosome 17p, which contains the p53 tumor suppressor gene, has been frequently detected in carcinoma in situ and high-grade, muscle-invasive tumors (1, 2). Since 9q alteration occurs in the early stages of TCC, this suggests that the inactivation of a suppressor gene on chromosome 9q is associated with the onset of TCC. A panel of region-specific probes spanning the length of chromosome 9 has demonstrated that the minimum common region that retains a deleted segment is localized between 9pl2 and 9q34.1 (3-5).

A gelsolin gene that encodes a $Ca2^{+}$ -binding and actin-regulatory protein has been mapped onto chromosome 9q33 (6, 7), whereas many biochemical and cytological studies have demonstrated that gelsolin regulates the length of actin filaments by severing and nucleating them and plays an important role in encouraging cell motility. The function of gelsolin has been modulated by $Ca2^{+}$ and PIP2 (8), whereas we ourselves have reported that a mouse gelsolin gene with a point mutation (His 321) revealed a tumor suppressor potential against NIH/3T3 cells transformed by a human activated H-ras oncogene (9). In the present study, we examined the gelsolin expression in human bladder cancers and the tumor suppressor activity of gelsolin upon a human bladder cancer cell line.

Materials and Methods

Bladder Cancer Cell Lines and Tissues. Six human urinary bladder cancer cell lines (TCCs) were cultured in DMEM supplemented with 10% FCS and 0.03% glutamine: EJ and T24 (provided by the Japanese Cancer Research Resources Bank); MGH-UI (provided by Dr. Y. Hashimoto, Tohoku University, Sendai, Japan); UMUC-2 and UMUC-6 (provided by Dr. B. Grossman, University of Michigan, Ann Arbor, MI); DAB-1 (established by Drs. Demura and Asano, Department of Urology, Hokkaido University School of Medicine, Sapporo, Japan). The cultures were maintained at 37'C in an atmosphere of 5% C02' Bladder cancer tissues were obtained either by transurethral operation or by radical cystectomy and stored at -80'C. Cancer tissues were staged according to the American Joint Committee on Cancer staging system and graded

according to the WHO classification. Eighteen TCC cancer tissues (stages and nos. of patients: Ta, 2; T1a, 3; T1b, 8; T2, 5; grades and nos. of patients: G1, 3; G2, 11; G3, 4) were available, and normal bladder mucosa and transitional cells derived from normal individuals were also used as a normal control.

Plasmids. We used a gelsolin expression vector, the human authentic gelsolin expression vector LKCG (kindly provided by Dr. D. J. Kwiatkowski, Harvard University, Boston, MA) and the mouse authentic gelsolin expression vector LK319 (kindly provided by Dr. C. W. Dieffenbach, Uniformed Services University of the Health Sciences, Bethesda, MD), described previously (9, 10). These vectors were derived from pH β APr-1-neo (LK444), which contained a G418 resistant marker. A plasmid pHRL83-BR (provided by Dr. F. Gunning, Stanford University and Veterans Administration Medical Center, Palo Alto, CA), which contained a human cardiac actin gene, was used a~ an internal control ·probe.

Northern, Western Blot Analysis, and Immunohistochemistry. Total cellular RNAS and cytoplasmic proteins were extracted as described previously (9, 11). For the collection of normal urinary transitional cells, fresh human normal bladder mucosa from the operative tissues of the epithelial layer alone were mechanically divided, minced, homogenized, and treated in the same way as the above (12). Northern and Western blot analyses were carried out as described previously (9, 11). A 2.4-kb BamHI-HindIII fragment of LK319 was used as a specific probe for gelsolin mRNA, while an 8.0-kb BamHI-EcoRI fragment of pHRL83-BR, which contained a human cardiac actin gene, was used as the internal control probe. Anti-human gelsolin monoclonal antibody (Biomakor, Rehovot, Israel) specifically bound M. 90,000 human gelsolin, and anti-mouse gelsolin polyclonal antibody prepared in our laboratory also bound M. 90,000 mouse gelsolin, whereas an anti-human actin mAb (Boehringer Mannheim Biochemica, Mannheim, Germany) was also used as the internal control antibody. The immunohistochemistry of gelsolin expressions was performed with the anti-human gelsolin antibody described above (13).

DNA Transfection. The transfection of the gelsolin expression vectors, LKCG, LK319, and LK444, into a human bladder cancer cell line UMUC-2 was performed by a lipofection procedure (Lipofectin; Bethesda Research Laboratories, Gaithersburg, MD), as described previously (9). To confirm the integration of gelsolin cDNA into the bladder cancer cell line, the total genomic DNA from each transfectant was analyzed by the

PCR amplification method. The sequences of PCR primers were as follows: 5'-ATAACCGAC-CCGTTACTT-3' (sense) and 5'-CCTTTrcTGAGGGTCCTT-3' (antisense). PCR products were electrophoresized in a 0.7% agarose gel and visualized by means of ethidium bromide staining. To confirm the expression of gelsolin in each transfectant, the total protein was analyzed by Western blot analysis, as described above.

Biological Assay. The colony-forming efficiency of each gelsolin transfectant in soft agar was carried out as described previously (9). Briefly, we inoculated each transfectant cells (1 x 104 cells/60-mm dish) in soft agar and observed the colony number at 3 weeks after inoculation. Statistical analysis was assumed by unpaired t test. To determine the tumorigenicity of each transfectant, 3-week-old nude mice were injected s.c. with 3 x l06 cells. Four weeks after injection, we observed the appearance of a palpable tumor at the site of the injection.

Results

Expressions of Gelsolin in Bladder Cancer Cell Lines and Tissues. We performed a Northern blot analysis to examine the expression of gelsolin mRNA in six human bladder cancer cell lines. The intensity of an approximately 2.4-kb single band of gelsolin transcripts was low or at undetectable levels in all six bladder cancer cell lines in comparison with that found in normal bladder mucosa (Fig. lA). Western blot analysis also revealed that when all six bladder cancer cell lines were compared with control normal bladder mucosa, they showed extremely low or undetectable expressions of gelsolin (Fig. 1B). Four bladder cancer tissues (BT-1, BT-7, BT-9, and BT-17) expressed gelsolin at a level similar to that found in normal control bladder mucosa, whereas 14 bladder cancer tissues (BT-2, BT-3, BT-4, BT-5, BT-6, BT-8, BT-10, BT-1 1, BT-12, BT-13, BT-14, BT-15, BT-16, and BT-18) expressed a markedly low level of gelsolin expression (Fig. IC). We then examined the localization of gelsolin by immunohistochemistry about BT-1, BT-7, BT-9, and BT-17. In normal urinary transitional cells and the bladder mucosa of noncancerous patients, gelsolin antibody stained the cytoplasm diffusely and intensively (Fig. 2, A and C). In contrast, neither bladder cancer cells (EJ) nor tissues (BT-17) responded to gelsolin antibody (Fig. 2, B and D). Only the

stromal cells, including the muscularis mucosa, the smooth muscle layer, and the cells surrounding the vessels in tumor nests, responded positively to gelsolin antibody in BT-17 cancer tissues (Fig. 2D). We have also observed similar staining of gelsolin in BT-1, BT-7, and BT-9 cancer tissues, except for their cancer cells like BT-17. These results confirmed that gelsolin expression is also reduced in BT-1, BT-7, BT-9, and BT-17 tumor cells.

Isolation of Gelsolin Transfectants. We transfected LKCG, LK319, and LK444 into UMUC-2 cells and isolated the UMUC-2 clones with a stable expression of gelsolin; these were the LKCG transfectants: UCG-a, UCG-b; LK319 transfectants: U319-a, U319-b; and LK444 transfectants: Uneo-a, Uneo-b. We used Western blot analysis to examine the expression of gelsolin and PCR to observe the integration of gelsolin gene (data not shown) in two clones of each transfectant. UCG clones strongly expressed human gelsolin in comparison with the untransfected UNUC-2 cells or Uneo control clones (Fig. 3A). U319 clones also intensively expressed the mouse gelsolin, but no staining of mouse gelsolin was exhibited in untransfected UNUC-2 cells or in Uneo control clones (Fig. 3A). We found that the cytoplasms of human authentic gelsolin-transfected cells (UCG-a and UCG-b) were elongated with filament-like protrusions in comparison with control vector transfectant (Uneo-a and Uneo-b) cells (Fig. 3B). The growth of gelsolin transfectant clones was slower, and their saturation density was low when compared with the control clones (data not shown).

Biological Assays. To examine the tumor suppressor activity of gelsolin, we tested its colony-forming efficiency in soft agar and its tumorigenicity in nude mice. All the UCG and U319 clones exhibited significantly lower colony-forming efficiency than did the Uneo control clones (as measured by unpaired t test; $P < 0.01$; Table 1). Furthermore, two UCG and U319-a clones showed no tumorigenicity in nude mice, whereas the U319-b clone lost tumorigenicity in three of the five nude mice (Table 1). We confirmed that two of five U319-b tumors in nude mice had reduced mouse gelsolin expression com-pared with in vitro U319 clones by Western blotting (data not shown). On the other hand, two Uneo control clones grew progressively in all of the nude mice,

Discussion

The multistage model of carcinogenesis proposes the growth of consecutive genetic abnormalities in oncogenes and tumor suppressor genes during tumor progression. Fearon and Vogelstein (14) have proposed this model for colorectal carcinogenesis. Probably, TCCs also pass through a similar course of genetic alteration of colorectal carcinogenesis in the development of human bladder carcinogenesis. Alterations of tumor suppressor genes, such as the mutations of p53, Rb, and DCC genes, are associated with the progression of invasive bladder cancers (1, 2). However, current concepts for the molecular basis of human bladder cancer are still indeterminate. Somatic cell hybrid analyses of genetic alterations in human bladder cancer have shown allelic losses, as well as deletions or rearrangements involving chromosomes 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 17, and 18 (1-5). There is also a loss of chromosome 9, especially allelic losses of chromosome 9q, and this loss is one of the most frequent genetic alterations in low-grade, low-stage bladder cancers. Previous analyses have suggested that one or more putative tumor suppressor genes on chromosome 9 have been mapped onto chromosome 9pl2 to 9qter (1, 3-5), whereas MTS-1 has been identified as a tumor suppressor gene on chromosome 9p21 (15). The inactivation of putative tumor suppressor genes on chromosome 9q may lead to abnormal uroepithelial proliferation and differentiation in the early stages of bladder carcinogenesis (1-5), but the presence of tumor suppressor genes on chromosome 9q has not yet been determined.

Gelsolin is a $Ca2^{+}$-binding and actin-regulatory protein, and the gene that encodes gelsolin is present in the human chromosome 9q33. Variations in gelsolin expression affect major cytoskeletal changes during differentiation and carcinogenesis. A diminished expression of gelsolin has been shown to be characteristic of a variety of trans-formed cells (9, 10). We have previously reported that gelsolin expression is significantly low in murine fibroblasts transformed by H-ras oncogene (10). In human fibroblasts and epithelial cells trans-formed with SV40 virus, gelsolin is one of the most striking down-regulated markers of the transformed state (16). Moreover, we have also observed a low or undetectable expression of gelsolin in seven of the eight gastric carcinoma cell lines (12), whereas there have been similar reports of gelsolin expression in human breast cancers and colon cancers (17, 18). In this present study, we showed

the reduced or undetectable gelsolin expression in 6 all bladder cancer cell lines and 14 of the 18 bladder cancer tissues (77.8%) compared with normal control bladder mucosa. There was no distinct correlation between tumor stage or grade and gelsolin expression. This could be because chromosome 9q alteration is an early event during carcinogenesis. On the other hand, gelsolin expression has been seen to increase during the differentiation of embryonal cells, although the expression of gelsolin has not been detected in murine undifferentiated embryonal carcinoma cell lines (19). When a 50-fold increase of gelsolin mRNA and protein levels has been induced by tetradecanoylphorbolacetate in the myeloid cell lines, U937 and HL60, it has been found that the differentiation of these cell lines causes them to develop into macrophage-like cells (20). Similarly, a 7-fold increase of gelsolin expression treated with trichostatin A in bladder cancer cell line T24 has caused morphological changes in the cytoskeletal elongation with protrusions and the reappearance of actin stress fibers (21). Our present study has demonstrated that the overexpression of gelsolin causes flatter morphological changes, as well as a growth disadvantage and a loss of tumorigenicity in the bladder cancer cell line UMUC-2. Furthermore, a region of the gelsolin gene has been re-ported to show loss of heterozygosity in human small cell lung cancer and medulloblastoma (22, 23). These observations suggest that the overexpression of gelsolin is essential for the differentiation of certain cancer cells and that the gelsolin gene is a candidate for the role of the tumor suppressor gene on the human chromosome 9q.

Cytoskeletal disorganization that disrupts actin filament formation is one of the major characteristics of a cancer cell. When a mutant β-actin gene was transfected into human normal fibroblasts, it functioned as a proto-oncogene and induced the transformation of these 1: cells (24). On the other hand, several reports have suggested that the overexpression of such actin-regulatory proteins as vinculin, α-actinin, and tropomyosin have shown a tumor suppressive function (25- 1: 27). We have demonstrated previously that mouse mutated gelsolin is able to suppress H-ras-transformed NIH/3T3 fibroblasts and human 1: authentic gelsolin may, if expressed at increased levels, have a suppressive potential against cancer (9). In our present expression and 1, transfection study, we have confirmed the tumor suppressive function of human or mouse gelsolin in the human bladder cancer cell line 1 UMUC-2.

Concerning the suppressive mechanism, there are several possibilities. Because gelsolin regulates the actin-filament length, and because gelsolin is able to bind to PIP2

and can inhibit PIP2 hydrolysis by PLCγ in vitro (8, 28), we speculate that the tumor suppressive 1 function of gelsolin is not only provoked by the interaction of its 1 cytoskeletal organization but also by the inhibition of signal transduction through the action of phosphoinositides. Our current study suggests that an alteration of the gelsolin gene may be associated with bladder cancer carcinogenesis. We are now analyzing the mechanism 2 of the down-regulation of gelsolin and how it may be applied in gene therapy against the human bladder cancer.

References

1. Kroft, S. H., and Oyasu, R. Biology of disease, Urinary bladder cancer: mechanisms of development and progression. Lab. Invest., 71.· 158-174, 1994.

2. Spruck, C. H., Ohneseit, P., Gonzalez-Zulueta, M., et al. Two molecular pathways to transitional cell carcinoma of the bladder. Cancer Res., 54: 784-788, 1994.

3. Linnenbach, A. J., Pressler, L. B., Seng, B. A., Kimmel, B. S., Tomaszewski, J. E., and Malkowicz, S. B. Characterization of chromosome 9 deletions in transitional cell carcinoma by microsatellite assay, Hum. Mol. Genet., 2.· 1407-1411, 1993.

4. Miyao, N., Tsai, Y. C,, Lerner, S. P,, Olumi, A. F., Spruck, C. H., Gonzalez Zulueta, M., Nichols, P. W., Skinner, D. G., and Jones, P, A. Role of chromosome 9 in human . bladder cancer. Cancer Res., 53: 4066-4070, 1993.

5. Orlow, I., Lianes, P., Lacombe, L., Dalbagni, G., Reuter, V. E., and Cordon-Cardo, C., Chromosome 9 allelic losses and microsatellite alterations in human bladder tumors. Cancer Res., 54.· 2848-2851, 1994.

6. Kwiatkowski, D, J., Westbrook, C. A., Bruns, G. A. P., and Morton, C. C. Localization of gelsolin proximal to ABL on chromosome 9. Am. J. Hum. Genet,, 42: 565-572, 1988. '

7. O'Brien) S. J., Womack, J. E., Lyons, L. A., Moore, K. J., Jenkins, N. A., and Copeland, N. G. Anchored reference loci for comparative genome mapping in mammalians. Nat. Genet., 3,· 103-112, 1993.

8. Janmey, P. A., and Stossel, T. P. Modulation of gelsolin function by phosphatidyl-inositol 4,5-bisphosphate. Nature (Lond.), 325: 362-364, 1987.

9. Mullauer, L., Fujita, H., Ishizaki, A., and Kuzumaki, N. Tumor-suppressive function of mutated gelsolin in ras-transformed cells. Oncogene, 8: 2531-2536, 1993.

10. Mullauer, L., Fujita, H., Suzuki, H., Katabami, M., Hitomi, Y., Ogiso, Y., and Kuzumaki, N. Elevated gelsolin and a-actin expression in a flat revertant R1 of Ha-ras oncogene-transformed NIH/3T3 cells. Biochem. Biophys. Res. Commun,, 171.· 852-859, 1990.
11. Fujita, H,, Suzuki, H., Kuzumaki, N., Mullauer, L., Ogiso, Y., Oda, A., Ebisawa, K., Sakurai, T., Nonomura, Y., and Kijimoto-Ochiai, S. A specific protein, p92, detected in flat revertants derived from NIH/3T3 transformed by human activated c-H-ras oncogene. Exp. Cell Res., 186: 115-121, 1990.
12. Moriya, S., Yanagihara, K., Fujita, H., and Kuzumaki, N. Differential expression of HSP90, gelsolin and GST-7T in human gastric carcinoma cell lines. Int. J. Oncol,, 5.-1347-1351, 1994.
13. Nakajima, K., Oda, A., Kobayashi, H., Katho, H., Kuzumaki, N., and Tanabe, T. Immunohistochemical study of ras p21 expression in human gastric cancer and benign lesions. Oncology, 46: 260-265, 1989.
14. Fearon, E. R., and Vogelstein, B. A genetic model for colorectal tumorigenesis, Cell, 61: 759-767, 1990.
15. Spruck, C. H,, Gonzalez-Zulueta, M., Shibata, A,, Simoneau, A. R., Lin, M. F., Gonzales, F., Tsai, Y. C., and Jones, P. A. p16 gene in uncultured tumors, Nature (Lond.), 370: 183-184, 1994.
16. Vandekerckhove, J., Bauw, G., Vancompernolle, K., Honore, B., and Celis, J. Comparative two-dimensional gel analysis and microsequencing identifies gelsolin as one of the most prominent down regulated markers of transformed hurnan fibroblast and epithelial cells, J. Cell Biol., 111.· 95-102, 1990.
17. Chaponnier, C., and Gabbiani, G. Gelsolin modulation in epithelial and stromal cells of mammary carcinoma. Am. J. Pathol., 134: 597-603, 1989.
18. Porter, R. M,, Holme, T. C., Newman, E. L., Hopwood, D., Wilkinson, J. M,, and Cuschieri, A. Monoclonal antibodies to cytoskeletal proteins: an immunohistochemical investigation of human colon cancer. J. Pathol., 170: 435-440, 1993.
19. Dieffenbach, C. W., SenGupta, D. N., Krause, D,, Sawzak, D., and Silverman, R. H. Cloning of murine gelsolin and its regulation during differentiation of embryonal carcinoma cells. J. Biol. Chem., 264: 13281-13288, 1989.
20. Kwiatkowski, D. J. Predominant induction of gelsolin and actin-binding protein during myeloid differentiation. J. Biol. Chem., 263: 13857-13862, 1988.

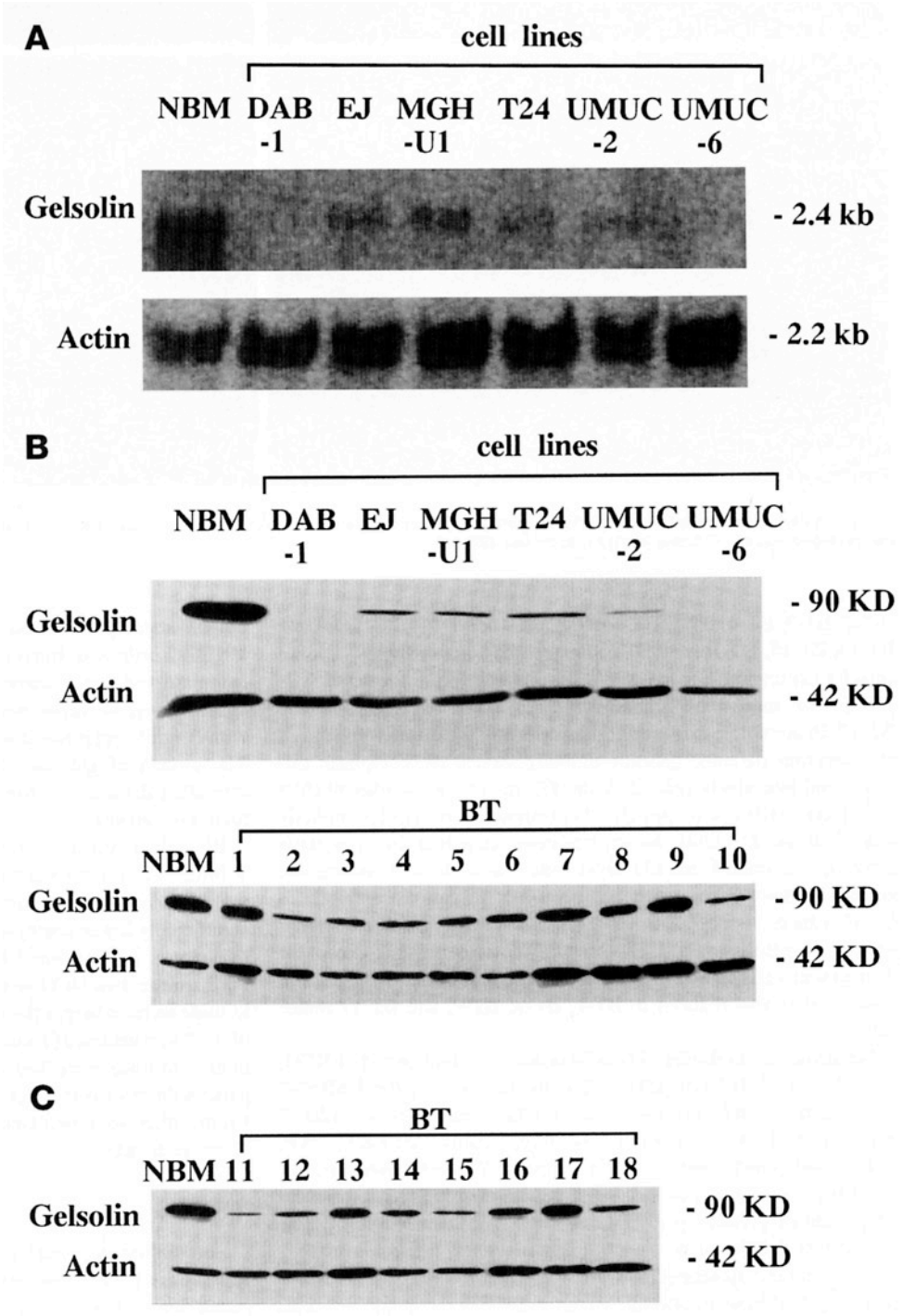
21. Hoshikawa, Y., Kwon, H. J., Yoshida, M., Horinouchi, S., and Beppu, T. Trichostatin A induces morphological changes and gelsolin expression by inhibiting histone deacetylase in human carcinoma cell lines. Exp. Cell Res., 214 189-197, 1994.
22. Merlo, A., Gabrielson, E., Mabry, M., Vollmer, R., Baylin, S. B., and Sidransky, D. Homozygous deletion on chromosome 9p and loss of heterozygosity on 9q, 6p and 6q in primary human small cell lung cancer. Cancer Res,, 54: 2322-2326, 1994.
23, Albrecht, S., von Deimling, A., Pietdch, T., Giangaspero, F,, Brandner, S., Klelhues, P., and Wiestler, O. D. Microsatellite analysis of loss of heterozygosity on chromosomes 9q, 11p and 17p in medulloblastomas. Neuropathol. Appl. Neurobiol., 20, 74-81, 1994.
24. Kakunaga, T. Mutation in genes cording for actin and its proteins in the neoplastic cell, Metabolism, 21: 763-773, 1984,
25. Fernandez, J. L. R., Geiger, B., Salomon, D., Sabanay, I,, Zoller, M., and Ben-Ze'ev, A. Suppression of tumorigenicity in transformed cells after transfection with vinculin CDNA. J. Cell Biol., 119: 427-438, 1992.
26. Gluck, U., Kwiatkowski, D. J., and Ben-Ze'ev, A. Suppression of tumorigenicity in simian virus 40-transformed 3T3 cells transfected with a-actinin CDNA. Proc. Natl. Acad. Sci. USA, 90: 383-387, 1993.
27. Prasad, G. L., Fuldner, R. A., and Cooper, H. L. Expression of transduced tropomyosin I cDNA suppresses neoplastic growth of cells transformed by the ras oncogene. Proc. Natl. Acad, Sci. USA, 90,' 7039-7043, 1993.
28. Banno, Y., Nakashima, T., Kumada, T., Ebisawa, K., Nonomura, Y., and Nozawa, Y. Effects of gelsolin on human platelet cytosolic phosphoinositide-phospholipase C isozymes. J. Biol. Chem., 267: 6488-6494, 1992.

Figure legends:

Fig. 1. A, expression of gelsolin mRNAs (Northern blot analysis) in human bladder cancer cell lines. B, expression of gelsolin proteins (Western blot analysis) in human bladder cancer cell lines. C, expression of gelsolin proteins (Western blot analysis) in human bladder cancer tissues.

Fig. 2. Immunohistochemical detection of human gelsolin expression (x 400). A, normal human urinary transitional cells in urine. B, human bladder cancer cell line (EJ). C, normal human bladder mucosa. D, human bladder cancer tissue (BT-17).

Fig. 3. A, expression of gelsolin proteins (Western blot analysis) in human or mouse gelsolin transfectants (UCG or U319) compared with untransfected UMUC-2 and control clones (Uneo). B, morphological feature (Giemsa staining, x 200) of control vector transfectant (Uneo-a) and human authentic gelsolin transfectant (UCG-a).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Gelsolin
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cell lines
NBM
DAB -1
EJ
MGH -U1
T24
UMUC -2
UMUC -6
Gelsolin
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Actin
- 42 KD
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- 90 KD
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Fig. 1

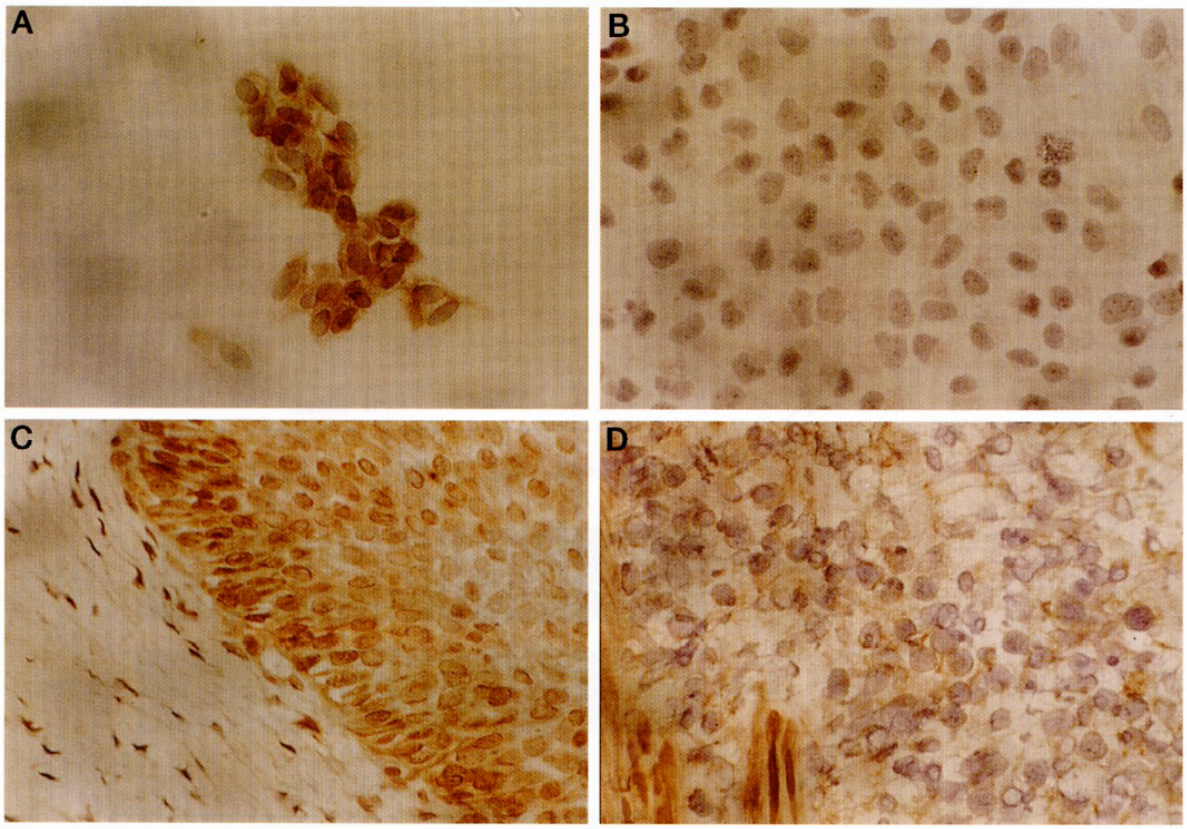

Fig. 2

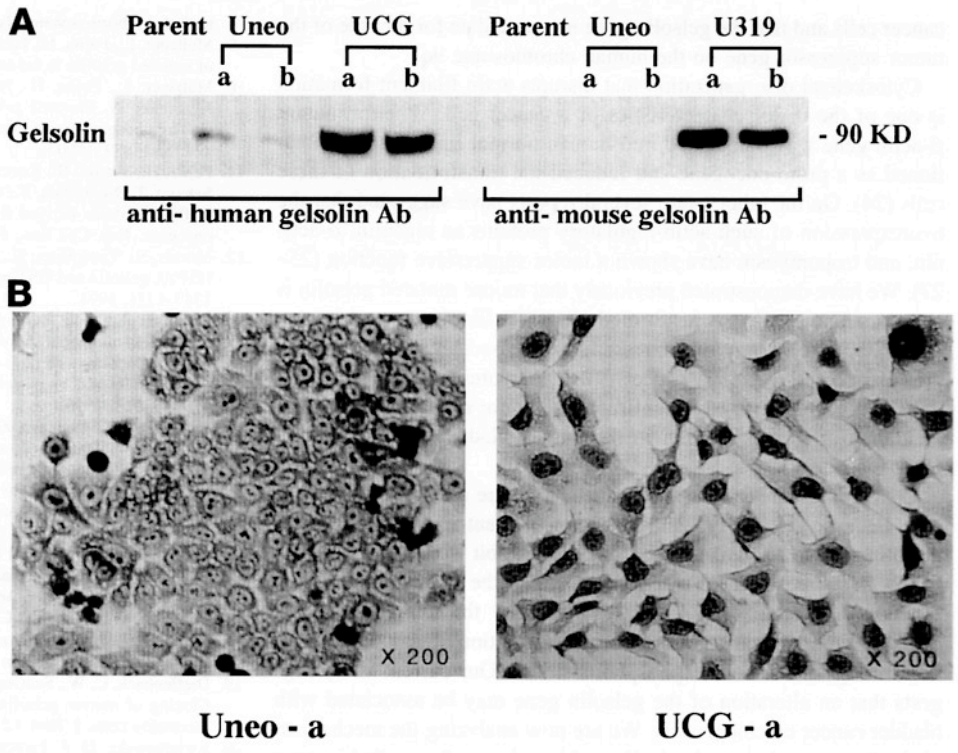
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Fig. 3

Table 1 *Colony-forming efficiency in soft agar and tumorigenicity in nude mice of human urinary bladder cancer cell line (UMUC-2) transfected with human (LKCG) or mouse (LK319) authentic gelsolin expression vectors or control vector (LK444)*

Cell clones	Transfected genes	Colony-forming efficiency (%) (mean ± SD)	Tumor incidence
UCG-a	LKCG	11.2 ± 1.7[a]	0/5
UCG-b	LKCG	18.4 ± 1.6[a]	0/5
U319-a	LK319	12.1 ± 1.2[a]	0/5
U319-b	LK319	18.2 ± 1.7[a]	2/5
Uneo-a	LK444	26.8 ± 0.7	4/4
Uneo-b	LK444	24.0 ± 2.4	3/3

[a] Unpaired *t* test: $P < 0.01$ (in comparison with Uneo-a and Uneo-b).