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Expression of Transfected Mutant β-Actin Genes: Transitions toward the Stable Tumorigenic State

JOHN LEAVITT,[1]* SUN-YU NG,[1] MADHU VARMA,[1] GERALD LATTER,[1] STEPHEN BURBECK,[1] PETER GUNNING,[1]† AND LARRY KEDES[2]

Armand Hammer Cancer Research Center, Linus Pauling Institute of Science and Medicine, Palo Alto, California 94306,[1] and The MEDIGEN Project, Department of Medicine, Stanford Medical School, and Veterans Administration Medical Center, Palo Alto, California 94304[2]

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Mutant human β-actin genes were introduced into normal human (KD) fibroblasts and the derivative cell line HuT-12, which is immortalized but nontumorigenic, to test their ability to promote conversion to the tumorigenic state. Transfected substrains of HuT-12 fibroblasts that expressed abundant levels of mutant β-actin (Gly-244 → Asp-244) produced subcutaneous tumors in athymic mice after long latent periods (1.5 to 3 months). However, transfected substrains of KD fibroblasts retained their normal finite life span in culture and consequently were incapable of producing tumors. Substrains of HuT-12 cells transfected with the wild-type β-actin gene and some transfected strains that expressed low or undetectable levels of mutant β-actin did not produce tumors. Cell lines derived from transfectant cell tumors always exhibited elevated synthesis of the mutant β-actin, ranging from 145 to 476% of the level expressed by the transfected cells that were inoculated to form the tumor. In general, primary transfectant cells that expressed the highest levels of mutant β-actin were more tumorigenic than strains that expressed lower levels. The tumor-derived strains were stable in tumorigenicity and produced tumors with shortened latent periods of only 2 to 4 weeks. These findings imply that the primary transfectant strains develop subpopulations of cells that are selected to form tumors because of their elevated rate of exogenous mutant β-actin synthesis. Actin synthesis and accumulation of γ-actin mRNA from the endogenous β- and γ-actin genes were diminished in tumor-derived strains, apparently to compensate for elevated mutant β-actin synthesis and maintain the normal cellular concentration of actin. Synthesis of the transformation-sensitive tropomyosin isoforms was decreased along with mutant β-actin expression. Such modulations in tropomyosin synthesis are characteristically seen in transformation of avian, rodent, and human fibroblasts. Our results suggest that this mutant β-actin contributes to the neoplastic phenotype of immortalized human fibroblasts by imposing a cytoarchitectural defect and inducing abnormal expression of cytoskeletal tropomyosins.

Only once has conversion of normal diploid human fibroblasts into a stable tumorigenic cell strain by treatment of cultured cells with a chemical carcinogen been reported (11). In contrast, rodent fibroblasts are transformed into tumorigenic cells at high frequency with carcinogens (11) and cloned oncogenes (16, 30). Spontaneous transformations also often occur in rodent cell cultures (16), but never in human cell cultures.

We have characterized the four in vitro-transformed human fibroblast (HuT) strains (18) derived by Kakunaga (11) in detail and compared them with the normal progenitor strain KD in an attempt to learn how human and rodent fibroblasts differ in the process of neoplastic transformation (1, 3, 8, 9, 12–19, 22, 31). Only one of these four transformed human strains (HuT-14) is consistently tumorigenic. The other three strains (HuT-11, HuT-12, and HuT-13) exhibit many transformation-related properties, including "immortalization" and aneuploidy, but these cells reproducibly fail to produce tumors in athymic mice and do not show the alterations in tropomyosin isoforms consistent with the tumorigenic state (19). The tumorigenic HuT-14 cell line is distinguished from the other three HuT strains not only by its tumorigenicity, but also by its acquisition of a mutation in β-actin, the most abundant structural protein of the cytoskeleton (14, 15, 17, 18, 22, 31). Although a relationship of the β-actin mutation to neoplastic or tumorigenic transformation seemed unlikely, our recent results, reported in the accompanying paper (21), caused us to reconsider this possibility.

In that work we introduced cloned mutant β-actin genes (17, 22) into KD cells and into the nontumorigenic HuT-12 cell line (21). The results of those experiments demonstrated that expression of the singly-mutant β-actin at levels nearly equal to or greater than those of the endogenous actins uniformly resulted in morphological and cytoskeletal alterations. We examined the tumorigenic potential of the transfected KD and HuT-12 cell strains to answer the question of whether the induction of these new phenotypes by mutant β-actin expression was sufficient to affect the tumorigenic potential of these transfected cells. Furthermore, these experiments would test the hypothesis that a high level of mutant β-actin expression played a role in the process of neoplastic transformation manifested by the transition from parental KD cells to tumorigenic HuT-14 cells (14, 18). In this paper we report that HuT-12 cells that express mutant β-actin consistently form solid tumors in inoculated athymic (nude) mice after a long latent period before appearance of the solid tumor. Furthermore, this tumorigenic property is most pronounced in transfected HuT-12 cell lines expressing the highest levels of mutant β-actin. Cell lines derived from the tumors are stable in their ability to form tumors in that they formed large, fast-growing

* Corresponding author.
† Present address: Children's Medical Research Foundation, Camperdown, Australia 2050.

TABLE 1. Tumorigenesis frequency in athymic mice inoculated with transformed and transfectant HuT fibroblasts

Tumor cell line	Ratio, mutant β-actin/ wild-type β-plus γ-actin[a]	No. of cells inoculated per mouse[b]	Tumor incidence (no. of mice with tumors/no. inoculated)	Tumorigenesis latent period (mo)
Transformed strains				
HuT-11	0	10^7	0/4	
HuT-12	0	10^7	1/8	3
HuT-12T	0	10^7	0/8	
HuT-13	0	10^7	0/4	
HuT-14	1	10^6–10^7	80/80	0.3–0.6
Transfectant strains				
HuT-12neo2	0		0/3	
HuT-12wt1	0		0/4	
HuT-12wt2	0		0/4	
HuT-12sm2	0.31		1/4	3
HuT-12sm2T	0.60		3/3	0.3–0.8
HuT-12sm4	0.88		4/4	1.5–3
HuT-12sm4T1	1.95		4/4	0.3–0.6
HuT-12sm4T2	1.67		3/3	0.3–0.6
HuT-12sm4T3	1.42		ND[c]	0.3–0.8
HuT-12sm6	0.13		2/6	3–4
HuT-12sm6T	0.62		3/3	0.3–0.8
HuT-12sm7	0.25		0/3	
HuT-12sm8	0.44		1/4	3
HuT-12sm8T	0.64		3/3	0.3–0.8
HuT-12sm11	0.81		4/8	1.5–3
HuT-12sm11T	1.06		21/23	0.3–0.8

[a] Rates of synthesis of the [^{35}S]methionine-labeled actin polypeptides were determined by computerized microdensitometry of actins resolved in 2-D gel autoradiograms (19, 21).

[b] Transfectant strains were all inoculated at 5×10^6 per mouse.

[c] ND, Not determined.

tumors in greater than 90% of the inoculated mice with a characteristically short latent period of 2 to 4 weeks. Each tumor-derived cell line was found to have significantly increased its expression of mutant β-actin 145 to 476%. This expression was accompanied by a decrease in synthesis of the transformation-sensitive tropomyosin isoforms.

MATERIALS AND METHODS

Cell cultures. The KD strain of diploid human fibroblasts and the HuT cell lines have been described previously (15, 18, 19). Transfection procedures and derivation of the G418-resistant primary transfected cell strains used in this study are reported in the accompanying paper (21). All cell types were cultured in Dulbecco modified Eagle medium supplemented with 20% fetal calf serum. Growth and passaging of cells and labeling with [^{35}S]methionine, followed by examination of cellular protein synthesis by high-resolution two-dimensional (2-D) polyacrylamide gel electrophoresis (PAGE) have also been described previously (19). The autoradiograms of the resulting 2-D gels were analyzed by computerized microdensitometry by both the automated and manual procedures described previously (19). Northern blot analysis was performed as described previously (29).

Tumorigenesis testing. To test transfected cell strains for their ability to produce tumors, 5×10^6 cells (per test animal) were inoculated into BALB/c nude mice under the skin in the lower back. These mice were maintained in a germ-free barrier isolator made by Simenson Laboratories (Gilroy, Calif.). Mice were examined for solid, palpable tumors at the site of inoculation on a weekly basis. After their appearance, tumors were allowed to grow to at least 0.5 cm in diameter, but most tumors grew to at least 1 cm in diameter. The appearance of these tumors was the same as that of tumors produced by HuT-14 and HuT-14T cells (published previously [14]). If the tumor-bearing mouse did not die unexpectedly, tumors were excised and fixed for histologic examination or cells were cultured from the tumor tissue for further examination. To culture cells from tumors, the tissue was minced into small fragments and incubated in 2 ml of trypsin-EDTA solution in a culture dish for 10 min at 37°C. Then, 25 ml of culture medium was added to the trypsinized tumor fragments and incubated under normal cell culture conditions. During this initial culturing period, 600 μg of G418 per ml was added to ensure sterility and remove mouse cells that might contaminate the human tumor cell culture. Confluent monolayers developed in 2 to 4 days. These tumor-derived cell strains were then examined by protein profiling with 2-D PAGE, and cells were reinoculated into nude mice to retest tumorigenicity as shown in Table 1.

RESULTS

Tumorigenicity of HuT cell lines. Previous experiments failed to detect tumorigenicity among cells from the immortalized HuT-11, -12, and -13 cell lines. We repeated these control experiments by injecting large inocula of these cell lines into athymic mice. Table 1 summarizes the results of subcutaneous tumorigenicity tests conducted with these parental HuT strains. As expected, no tumors were detected in inoculations of HuT-11 or HuT-13 cells. However, one of eight athymic mice inoculated with 10^7 HuT-12 cells did produce a small subcutaneous tumor (0.5 cm diameter) after a 3-month latent period during which no tumor was discernible. However, this isolated event did not represent stable acquisition of tumorigenicity, because when this tumor-derived HuT-12T strain was retested for tumorigenicity, it failed to produce tumors (Table 1). Furthermore, the cell line HuT-12T derived from this lone tumor exhibited no alterations from the standard HuT-12 protein profile in 2-D gels. For example, HuT-12T cells showed the same "nontumorigenic mode" of tropomyosin isoform expression as the parental nontumorigenic HuT-12 strain, and they secreted copious amounts of fibronectin into the extracellular matrix. We conclude that the three strains HuT-11, -12, and 13 are nontumorigenic. This contrasts sharply with the uniform tumorigenic potential of the HuT-14 cell line (Table 1).

TABLE 2. Rates of synthesis of mutant β-actin and β-plus γ-actin in transfected human fibroblasts[a]

Strain	Total actin (%)	Endogenous β-plus γ-actin (%)	Exogenous mutant β-actin (%)
HuT-12	31.2	31.2	
HuT-12sm2	31.6	24.5	7.1
HuT-12sm2T	34.8	21.7	13.1
HuT-12sm4	27.5	14.4	13.1
HuT-12sm4T1	34.2	11.6	22.6
HuT-12sm6T	28.8	17.8	11.0
HuT-12sm8	26.8	19.0	7.8
HuT-12sm8T	30.0	18.3	11.7

[a] Disintegrations per minute in actin expressed as the percentage of the total in 600 abundant acidic polypeptides (21).

Growth properties of transfected cells. All individual transfected strains of KD cells (see Table 1 in reference 21) exhibited a finite life span in culture and terminated their growth between passage 9 and passage 17 in a pattern consistent with the expected life span of KD cells in culture (11). Futhermore, there was no apparent growth-inhibitory effect accompanying abundant expression of exogenous mutant β-actin (21). KDsm-5 cells were inoculated subcutaneously into three athymic nude mice (5×10^6 cells per mouse), but no tumors developed after 5 months. Abundant expression of transfected mutant β-actin in HuT-12 subclonal cells had no apparent effect on their characteristic doubling time of 21 h, which is unchanged from that of the parental HuT-12 strain.

Tumorigenicity of transfected HuT-12 cells. We selected a series of transfected HuT-12 cells that either lacked detectable synthesis of mutant β-actin or exhibited relatively low or relatively high levels of mutant β-actin synthesis (Table 1). Remarkably, five independently transfected strains which synthesized mutant β-actin as one of the most abundant cellular proteins (sm2, sm4, sm6, sm8, and sm11) produced nodular subcutaneous tumors in athymic mice which appeared as early as 1.5 months or as late as 4 months after cell implantation (Table 1). After their appearance these subcutaneous tumors grew to at least 0.5 cm in diameter within 4 weeks. These tumors were phenotypically similar to those produced by 2×10^6 HuT-14 cells 5 weeks after implanation (14). Transfectant cells that expressed mutant β-actin at a relatively low level (sm7) failed to produce tumors. Control cells, transfected only with the neomycin resistance gene (neo2) or the wild-type β-actin gene (wt1 and wt2), also failed to form tumors.

Tumorigenic phenotype is stable. Since tumorigenic conversion of human fibroblasts is a rare occurrence, we took advantage of this finding to examine the stability of the tumorigenic phenotype. Secondary strains derived from the transfectant cell tumors (designated in Table 1 by the suffix T) were stably elevated in tumorigenic potential in that they formed subcutaneous tumors in 90 to 100% of the mice inoculated. In addition, the tumors that formed inoculations of most of these tumor-derived lines appeared in a considerably shorter interval (within 2 to 4 weeks) than the rather protracted tumor growth emergence of the original transfectant line.

Elevated expression of the exogenous mutant β-actin. The proteins synthesized by these tumor-derived lines were examined by 2-D PAGE. Surprisingly and without exception, each subline exhibited a markedly higher rate of synthesis and amount of mutant β-actin than the primary transfectant HuT-12 strain from which the tumor was formed (Table 1). For example, the primary transfectant strain HuT-12sm4, which exhibited the highest rate of synthesis of mutant β-actin after transfection (ratio of mutant β-actin to endogenous wild-type β- plus γ-actin, 0.9), expressed a 2.2-fold-higher rate of mutant β-actin in its tumor-derived strain HuT-12sm4T1. Two additional independent tumorigenesis events with HuT-12sm4 cells resulted in tumors composed of cells (HuT-12sm4T2 and -sm4T3) which exhibited a 1.9- or 1.6-fold-enhanced rate of mutant β-actin synthesis. Likewise, HuT-12sm11T, HuT-12sm8T, and HuT-12sm2T expressed 1.3-, 1.5-, and 1.9-fold-higher rates of mutant β-actin synthesis, respectively, than the primary tranfectant strain before subcutaneous implantation. Even the transfectant strain HuT-12sm6, which synthesized a relatively low amount of mutant β-actin, produced two tumors, one of which exhibited a 4.5-fold elevation in rate of mutant β-actin synthesis. Our assumption is that the athymic mice provided a selective environment for selection of a phenotype related to elevated expression of the mutant β-actin protein. The augmented expression of the mutant β-actin in these tumor-derived strains supports the notion that expression of this mutant β-actin is linked to the tumor-forming capacity of HuT strains.

Autoregulation of wild-type β and γ-actin expression in tumor-derived transfectant HuT-12 strains. In the accompanying paper (21) we demonstrated that abundant expression of the exogenous mutant β-actin gene in KD and HuT-12 transfectant strains did not lead to an increase in the total amount of actin per cell. In each transfected strain tested there was an equivalent reduction in rates of synthesis of wild-type β- and γ-actin to compensate for additional synthesis of actin from the exogenous mutant β-actin gene. We reexamined this autoregulatory phenomenon in the tumor-derived strains HuT-12sm2T, -sm4T1, and -sm8T which exhibited 1.9-, 2.2-, and 1.5-fold elevations, respectively, in the rate of mutant β-actin synthesis relative to the rates in the primary transfectant strains (Table 1). There was a concomitant reduction in rates of β-and γ-actin synthesis in each of the tumor-derived strains (Table 2), although the total amount of actin in each tumor-derived strain increased slightly. This result provides further evidence that total actin synthesis must be regulated so that expression of an exogenous actin gene inhibits endogenous actin synthesis to maintain an optimal homeostatic concentration of cellular actin (21).

Autoregulation of γ-actin mRNA levels. The profound downregulation of endogenous β- and γ-actin synthesis in response to expression of the exogenous mutant β-actin gene raises the question of whether autoregulation occurs at the level of transcript accumulation. Although we cannot discriminate between the endogenous and exogenous β-actin mRNAs, we can measure endogenous γ-actin mRNA levels by using a 3′ untranslated region γ-actin-specific DNA probe (29). We therefore prepared RNA from the cell lines showing the greatest reduction in endogenous actin synthesis and measured total β-actin (mutant plus wild type) and endogenous γ-actin mRNA levels relative to that in the parental HuT-12 cell line and KD cells. A small elevation of β-actin mRNA levels was apparent in the three transfectant lines (Fig. 1A, lanes 4 to 6) compared with that in HuT-12, HuT-14, and KD cells (Fig. 1A, lanes 1 to 3). Densitometry revealed a 20% increase in the β-actin mRNA level in the three transfectant lines over that seen in the parental HuT-12 cells (Table 3). This paralleled the increase seen in total β-actin (mutant plus wild type) synthesis in these three cell lines (Table 3).

The three transfectant cell lines showed a clear decrease in their level of γ-actin mRNA in comparison with the HuT-12 cell line (Fig. 1B). Densitometric quantification revealed that the level of γ-actin mRNA was reduced to 90, 30, and 50% of the HuT-12 level in HuT-12sm4, HuT-12sm4T, and HuT-12sm11T, respectively (Table 3). This closely paralleled the decrease in γ-actin synthesis in the two tumor-derived cell lines (HuT-12sm4T and HuT-12sm11T), but was less than the reduction of γ-actin synthesis in the transfectant HuT-12sm4 (Table 3). In particular, whereas γ-actin mRNA levels were reduced by 70% following passage of HuT-12sm4 cells through a tumor to yield the HuT-12sm4T line, γ-actin protein synthesis was reduced by only 20% (Table 3). A similar anomaly was also noted with the KD cells. Whereas the relative synthesis of both β- and γ-actin and the level of β-actin mRNA were virtually identical between KD,

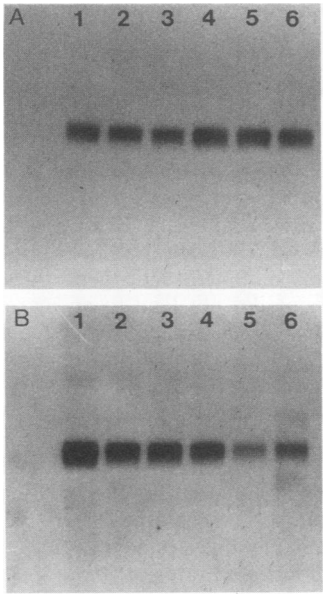

FIG. 1. Autoradiograms of total RNA transfer blots hybridized with [^{32}P]DNA probes derived from isotype-specific β-actin (A) or γ-actin (B) gene 3′ untranslated region sequences (16, 29). Source of total RNA: lane 1, KD; lane 2, HuT-12; lane 3, HuT-14; lane 4, HuT-12sm4; lane 5, HuT-12sm4T; lane 6, HuT-12sm11T.

HuT-12, and HuT-14 cells, the KD cells accumulated 50% more γ-actin mRNA than the two HuT cell lines (Table 3). Thus, the autoregulatory mechanism responsible for decreased γ-actin synthesis at least partially operated at the level of mRNA accumulation. However, in KD and HuT-12sm4 cells, the level of γ-actin synthesis was less than would be expected from the mRNA levels. This suggests that γ-actin synthesis can be regulated to a certain extent independently of mRNA levels. The same conclusion can also be drawn from our previous demonstration that a 10-fold increase in the γ-actin mRNA level in a Rat-2 cell line yielded only a threefold increase in γ-actin protein synthesis (16).

Modulation of tropomyosin isoform expression in transfectant diploid KD cells. Major changes in the stoichiometry of cytoskeletal proteins often accompany neoplastic transformation (6, 16, 19, 20, 24, 25, 32). For example, the cytoskeletal disruptions that accompany transformation of a number of rodent and avian cell lines by viruses or oncogenic DNA fragments is associated with the apparent cessation of α-actin gene expression (6, 16, 32), modulation of β- and γ-actin synthesis (6, 16, 20), and major modulations of the relative levels of expression of six tropomyosin isoforms (4, 6, 10, 19, 24, 25). Similarly, the six tropomyosin isoforms of KD cells (Tm1 to Tm6) (19) are distinctly and differently modulated following transformation that results in either just immortalization or both immortalization and full tumorigenicity. The synthesis and accumulation of Tm1, Tm2, Tm3, and Tm6 are significantly decreased in HuT-14 cells. In contrast, HuT-12 cells, which are immortalized but rarely tumorigenic and lack the mutant β-actin, exhibit a less pronounced decrease of Tm1 and Tm6 and a contrasting increase in the synthesis and accumulation of Tm3 (19).

Whether these alterations in cytoskeletal proteins following transformations contribute to the increased tumorigenicity or are simply a result of immortalization, the stoichiometry of cytoskeletal proteins is maintained in a new stable balance. The ability to introduce and express redundant copies of the wild-type, singly-mutant, and triply-mutant cytoskeletal β-actin genes into normal diploid KD cells allowed us to assess the impact of mutant β-actin synthesis on the expression of other cytoskeletal proteins independent of transformation or immortalization effects. The rates of synthesis of three of the six Tm isoforms of KD cells (Tm2, Tm3, and Tm6) (Fig. 2A) were greatly reduced in KDsm-5 cells (Fig. 2C) which, among the transfected diploid strains, had the highest rate of synthesis of exogenous β-actin with the single mutation (22). However, Tm isoform synthesis was not significantly altered in control transfectant strains which expressed either the exogenous triply-mutant β-actin, KDtm-2 (Fig. 2D), or that failed to express detectable levels of exogenous mutant β-actin following transfection, KDtm-1 (Fig. 2B) (22).

The data in Fig. 3 contrast the relative rates of Tm isoform synthesis after further subculturing of cell cultures expressing single-mutant β-actin, KDsm-5 (Fig. 3B), or triply-mutant β-actin, KDtm-2 (Fig. 3A). Synthesis of Tm2, Tm3, and Tm6 was greatly decreased, and synthesis of Tm1 was slightly downregulated in the KDsm-5 strain. By contrast, polypeptide a (a soluble protein of unknown identity [19]) and Tm5 were not greatly affected (compare also Fig. 2A and 2C). Polypeptide b, which may be the nuclear antigen cyclin (6, 19), was decreased nearly as much as Tm2 (see Fig. 2C). Tm2′ (19), which is probably derived from Tm2, was not detectable (see Fig. 2C).

We calculated the relative rates of synthesis of the Tm isoforms by two different methods of computerized microdensitometry. First we singled out 160 highly resolved, abundant polypeptides for manual measurement. Table 4 presents the relative rates of synthesis of the Tm isoforms and actin calculated as the percentage of the selected polypeptides. Nearly identical ratios between these polypeptides were obtained by automated measurement of the rates of 600 polypeptide species (not shown). A control substrain which was transfected with a neomycin resistance gene but failed to express the exogenous β-actin gene (KDtm-1 [21]) had Tm synthesis patterns almost indistinguishable from those of wild-type KD cells. Thus, the transfection and selection procedures do not themselves affect the patterns of protein synthesis. However, expression of the exogenous singly-mutant β-actin gene in KDsm-5 cells had an inhibitory effect on the expression of five of the six tropomyosin isoforms (Tm1, Tm2, Tm3, Tm4, and Tm6). Of further interest, these effects were not evident in a strain (KDtm-2 [21]) expressing the triply-mutant actin gene.

In KDsm-5, the rates of Tm2, Tm3, and Tm6 synthesis

TABLE 3. Relative actin mRNA levels and protein synthesis

Strain	Relative mRNA level[a]		Relative protein synthesis[b]	
	β[c]	γ	β[c]	γ
HuT-12	1.0	1.0	1.0	1.0
HuT-12sm4	1.2	0.9	1.2	0.5
HuT-12sm4T	1.2	0.3	1.4	0.4
HuT-12sm11T	1.2	0.5	1.3	0.5
HuT-14	0.9	1.0	1.0	1.0
KD	1.0	1.5	1.0	1.0

[a] mRNA levels were quantitated by densitometry of the autoradiograms shown in Fig. 1. The levels are expressed relative to the level in HuT-12 cells.
[b] Protein synthesis levels were measured as described in the text and are expressed relative to the level in HuT-12 cells.
[c] Sum of mutant plus wild-type β-actin.

were decreased 76, 89, and 74%, respectively, while Tm1 and Tm4 were decreased 41 and 46% respectively (Table 4). In contrast, synthesis of Tm5 was unaltered in KDsm-5. Such pronounced Tm isoform modulation was not observed for the three additional strains tested, KDtm-1 (Table 4), KDwt-3 (not shown), and KDtm-2 (Table 4). We examined the specificity of Tm modulation in KDsm-5 cells by comparing the relative modulation of the 160 highly resolved, matched polypeptides measured in the experiment in Table 4 between normal KD cells and different transfectant cell strains. A pairwise comparison of the rates of the 160 individual polypeptides was plotted in scatter plots (Fig. 4). The ratios between individual polypeptides in KDsm-5 and the same polypeptides in KD cells or their average rates of synthesis in the three control strains (KD, KDtm-2, and KDtm-1) demonstrate that of the 160 polypeptides, 6 discrete polypeptides, Tm1, Tm2, Tm3, Tm6, and polypeptides d and e (Fig. 2), were the most signficantly modulated. The other polypeptides measured were not modulated beyond the range of error indicated by the two control scatter plots (at the right of Fig. 4). We conclude that the decreased synthesis of specific isoforms of tropomyosin which bind tightly to wild-type actin is associated with a high level of synthesis of a stable but mutant β-actin in diploid KD cells.

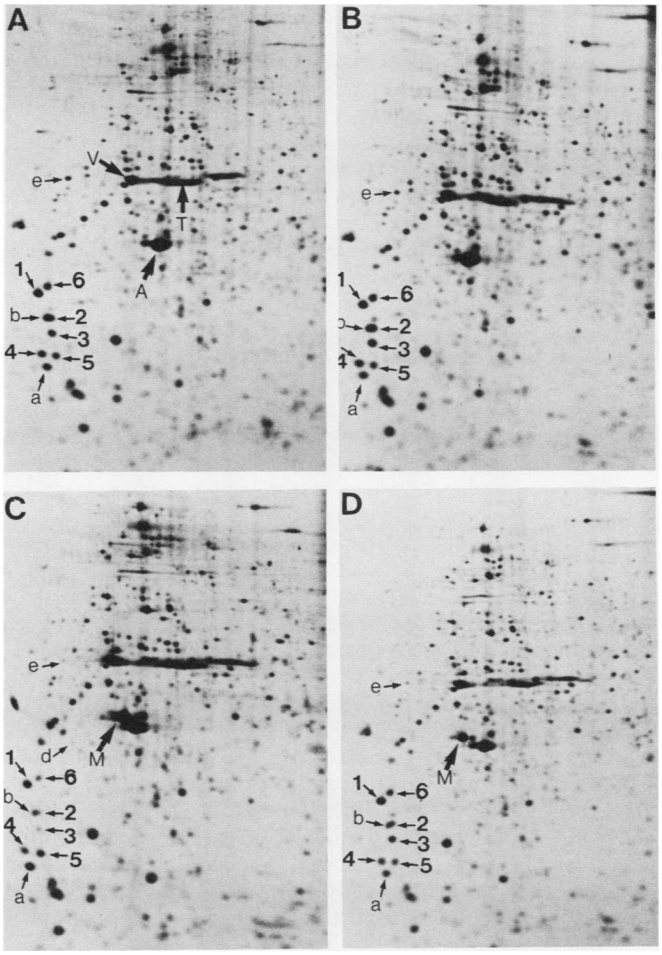

FIG. 2. Autoradiograms of [^{35}S]methionine-labeled polypeptides from normal KD fibroblasts and three transfectant subcolonial strains separated by high-resolution 2-D PAGE (19). (A) Normal KD fibroblast polypeptides; (B) transfectant G418-resistant subcolonial strain KDtm-1 passage 6, which does not express the exogenous triply-mutant β-actin gene; (C) transfectant strain KDsm-5 passage 6; (D) transfectant strain KDtm-2 passage 5. The major cytoskeletal polypeptides that are identified in panel A only are: A, normal β- and γ-actins (pI 5.3; 42,000 kilodaltons) (18, 31); V, vimentin (pI 5.1; 56,000 kilodaltons) (18); and T, α- and β-tubulin (18), which have formed poorly resolved horizontal streaks. M, Mutant β-actin (panels C and D). Polypeptides 1 through 6 are the six Tm isoforms (19). Polypeptide e was downregulated in KDsm-5 cells (panel C; see Fig. 4), and polypeptide d appeared only in KDsm-5 cells (panel C). Polypeptides a and b are discussed in the text.

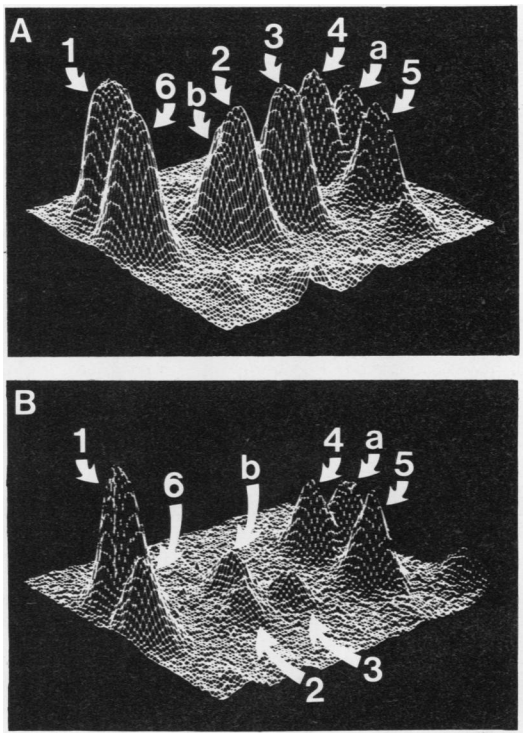

FIG. 3. Computer-generated three-dimensional graphs indicating the relative quantities (disintegrations per minute per pixel) of the six TM isoforms and neighboring polypeptides in 2-D gel autoradiograms (see Fig. 2) of KDtm-2 (A) and KDsm-5 (B) of [^{35}S]methionine-labeled polypeptides isolated from cells in passage 8 after transfection. The volume of the peaks reflects the relative quantified disintegration rates produced by these polypeptides after exposure of the 2-D gel patterns to film for 24 h.

The microdensitometry results in Table 4 were used to calculate the ratios (in [^{35}S]methionine residues) between the different Tm isoforms and actin (Table 5). In normal KD cells the ratio of Tm (combined isoforms) to actin (combined isoforms) was about 0.13. Previously we found this ratio to be 0.14 for both KD and HuT-12 cells (20), and a ratio of 0.12 was reported for rat REF-52 cells (6). The control strain KDtm-1 demonstrated a slightly elevated ratio of 0.16 (Table 5). In the mutant β-actin transfectant KDsm-5, this ratio decreased greatly to 0.05 (Table 5) due to a 68% reduction in the combined rate of synthesis of the four high M_r Tm isoforms (Tm1, Tm2, Tm3, and Tm6), a 54% reduction in Tm4, and a slight increase in the amount of total actin synthesis (Table 4).

We also measured relative Tm and actin isoform expression in another mutant β-actin gene transfected strain, KDsm-9 (21), at early and late passages after transfection with the singly-mutant β-actin. In the early passages, this strain is composed of a relatively minor subpopulation of cells which abundantly express the mutant β-actin (21).

TABLE 4. Relative rates of synthesis of tropomyosins and actins based on the sum of 160 reference proteins[a]

Polypeptide	Relative rate of synthesis (%)			
	Parental KD strain	Transfectant KD strain[b]		
		KDsm-5	KDtm-2	KDtm-1
Tm1	3.19	1.89 (0.59)	3.60 (1.13)	3.93 (1.23)
Tm6	1.71	0.45 (0.26)	1.35 (0.79)	1.59 (0.93)
Tm2	1.12	0.27 (0.24)	0.67 (0.60)	1.18 (1.05)
Tm3	1.91	0.21 (0.11)	1.45 (0.76)	1.34 (0.70)
Tm4	1.49	0.81 (0.54)	1.20 (0.81)	1.43 (0.96)
Tm5	0.95	1.02 (1.07)	0.93 (0.98)	1.04 (1.09)
All Tms	10.37	4.65 (0.45)	9.20 (0.89)	10.51 (1.01)
Tm1 + Tm2 + Tm3 + Tm6	7.93	2.82 (0.36)	7.07 (0.89)	8.04 (1.01)
Tm4 + Tm5	2.44	1.83 (0.75)	2.13 (0.87)	2.47 (1.01)
β- plus γ-actin	40.36	21.71 (0.54)	40.59 (1.01)	36.64 (0.91)
Mutant β-actin (stable form)[c]	—[d]	20.45 (0.51)	—	—

[a] The [^{35}S]methionine disintegrations per minute in each polypeptide species in 2-D gels were measured by the manual method of computerized microdensitometry (19).

[b] Transfectant cell strains are described in reference 21 and in the text. The fraction of the rates of polypeptide synthesis achieved by parental uncloned KD cells is shown in parentheses.

[c] The triply-mutant β-actin (Fig. 2D) was rapidly degraded (21) and therefore did not contribute significantly to the steady-state concentration of actin.

[d] —, Not present.

During continuous subculturing of this strain, the transfected cells expressing mutant β-actin eventually outgrow the transfected cells that fail to express mutant β-actin. In one of two independent demonstrations of this phenomenon, the rate of expression of the mutant β-actin slightly exceeded the rate of expression of the endogenous wildtype β- and γ-actin in the cell culture at passage 11 after subcloning (21). Examination of the cells with mutant β-actin-specific antibody (21) by indirect immunofluorescence indicated that the subpopulation of cells expressing mutant β-actin has increased from about 5% in passage 4 to about 90% by passage 11. We measured the relative rates of Tm and actin isoform expression to determine how the critical stoichiometry between the Tm isoforms and actin had changed as the mutant-β-actin-expressing cells became the predominant cell species in the culture. In KDsm-9 cells at passage 11 the pattern of Tm reduction was similar to that in KDsm-5 cells, although less pronounced (Table 5). Comparing passage 11 cells with passage 4 cells, there was a 30% reduction in total Tm isoforms relative to actin, which was due primarily to a 40% reduction of the high-M_r Tm isoforms (Tm1, Tm2, Tm3, and Tm6) relative to actin (Table 5). However, about 10% of the cells in this culture did not express mutant β-actin. Thus, the levels of Tm isoform synthesis in this experiment reflect the average of these two distinct subpopulations (21).

Modulation of tropomyosin isoform expression in transfected immortalized HuT-12 cells. Immortalized HuT-12 cells, compared with normal parental KD cells, exhibit a reduction in Tm1 and Tm6 and an elevation in Tm3 synthesis (19). This mode of Tm synthesis contrasts sharply with Tm synthesis in tumorigenic HuT-14 cells, which exhibit a more pronounced reduction in synthesis of Tm1, Tm2, and Tm6 and a slight reduction in synthesis of Tm3 (19). We were therefore interested to determined the effect of expression of mutant β-actin on Tm synthesis in HuT-12 cells. We examined tropomyosin synthesis in primary tranfectant strains and in the tumor-derived stains listed in Table 1 to determine whether Tm expression was altered in a pattern consistent with Tm expression in fully tumorigenic HuT-14 cells. Since the rate of β- and γ-actin synthesis in KD, HuT-12, and HuT-14 cells is essentially the same (15, 19) and since the combined rates of actin isoform synthesis in transfected KD and HuT-12 cells are essentially the same (Table 2) (21), we calculated the effect of mutant β-actin synthesis on Tm synthesis by determining the ratio of Tm isoforms to total actin by using manual microdensitometry.

Of the primary transfected strains, HuT-12sm4 was the most tumorigenic, producing tumors in all four mice inoculated (Table 1). Comparison of Tm expression between this primary transfectant strain and the parental strain HuT-12 revealed that synthesis of all six Tm isoforms was inhibited by 23 to 75% resulting in a 36% reduction of the total Tm-actin ratio (Table 6). In the fully tumorigenic strain HuT-12sm4T1, the ratios of Tm1, Tm2, Tm3, Tm4, and Tm6 to total actin were reduced still further, resulting in a 62% reduction in the total Tm-actin ratio (Table 6). Again, this reduction in the tropomyosin-actin ratio occurred without a significant increase in the total synthesis rate of actin (Table 2) (21).

HuT-12sm2 and HuT-12sm8 cells, which exhibited lower levels of mutant β-actin synthesis (21), showed less inhibition of Tm isoform synthesis (Table 6). However, tumor-derived strains produced from these primary transfectant strains exhibited both elevated rates of mutant β-actin synthesis and more pronounced reduction in total Tm synthesis. All five tumor-derived strains that were tested exhibited elevated mutant β-actin synthesis (Table 1) and reduced Tm synthesis as a consequence of passage through tumors, a matching reduction in endogenous β- plus γ-actin gene expression (Table 2), and full tumorigenicity with a short latent period (Table 1). These results suggest that reduction of Tm synthesis may be coordinated with a reduction of endogenous actin gene expression and a concomitant increase of mutant β-actin expression.

Induction of a new polypeptide by mutant β-actin. Synthesis of polypeptide d (M_r 40,000, pI 4.9) was induced in KD cells (see Fig. 6 and 2C) and HuT-12 cells (Fig. 5B and C) following high-level expression of the singly mutated β-actin, but not in control strains such as KDtm-2 or KDtm-1, which express the unstable triply mutated β-actin (Fig. 2D) or no mutant β-actin (Fig. 2B and 6C). This polypeptide was also found in HuT-14 cells (Fig. 5D) but not in HuT-12 cells (Fig. 5A), which lack a mutant β-actin, or in HuT-14T cells (data not shown), which express the triply-mutant β-actin (14). Polypeptide d was also expressed in HuT-12sm4 cells, but it was not detected in other transfected cell strains that expressed lower levels of mutant β-actin. We conclude that expression of the stable singly mutated β-actin at high levels results in induction of synthesis of this new polypeptide.

DISCUSSION

Contribution of mutant β-actin to the neoplastic phenotype of HuT-14 cells. The original observation of a mutant β-actin within the tumorigenic HuT-14 cells raised the question of whether the β-actin mutation could have contributed to the transformed phenotype of HuT-14 cells. By comparing KD, HuT-14, and singly mutant KD cell transfectants, we evaluated which changes in HuT-14 cells could have been generated by the mutation which occurred in β-actin.

The KDsm-5 and KDsm-9 cells display a transformed morphology similar in a number of respects but not identical to that of HuT-14. The major morphological difference is the characteristic cytoskeletal protrusions in the transfected KD

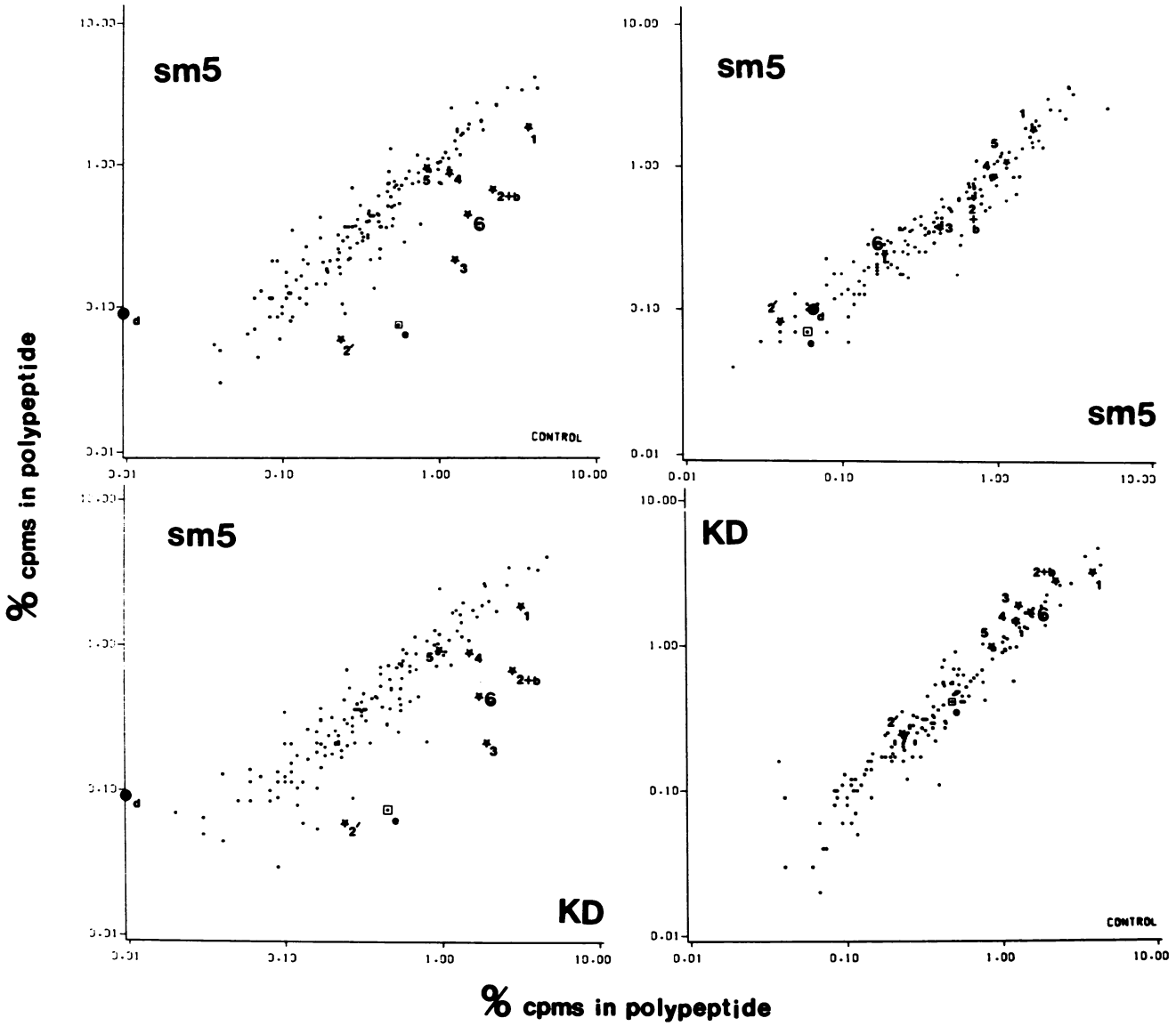

FIG. 4. Pairwise comparison in a radioactivity scatter plot of 160 polypeptide species (from Table 4). Each axis represents the radioactive disintegrations or average disintegrations per minute present in an individual polypeptide in total-protein 2-D gel autoradiograms (Fig. 2) from the cell strains indicated on the abscissa and ordinate axes. Control (cell strains) means that the average disintegrations from microdensitometry measurements of three cell strains, KD, KDtm-1, and KDtm-2, were used as the rate of expression of the polypeptide species. The upper and lower graphs on the left compare rates of synthesis of individual polypeptide species in KDsm-5 (Table 4) with either the three control transfectant strains (upper left) or untransfected KD cells alone (lower left). The KDsm-5 microdensitometry data were the average of measurements from two independent gel autoradiograms. The range of scatter of microdensitometry data due to error is indicated in the upper and lower graphs on the right. The upper right compares the microdensitometry measurements between two independent gels of polypeptides from KDsm-5. The lower right compares the microdensitometry measurements between untransfected KD cells and the average of the three control cell strains. ★, Tm isoform measurements; ⊡, polypeptide e measurements; ●, polypeptide d measurements.

cells (21). In addition, KDsm-5 cells continue to secrete normal amounts of fibronectin into the extracellular matrix in contact with the cytoskeleton (unpublished results), whereas HuT-14 cells are greatly diminished in this function (14). Thus, the mutant β-actin may produce a partial morphological transformation, but requires the action of other factors or events to cause a complete transformation characterized by a loss of actin cables and a reduction of fibronectin in the extracellular matrix.

The Tm phenotype in KDsm-5, KDsm-9, and the tumor-derived transfectant HuT-12 cells is similar to that observed in HuT-14 cells and other tumorigenic cells. A number of workers have demonstrated that after tumorigenic transformation of avian (10), rodent (4, 6, 24), and human (19) cells, a consistent downregulation of strongly binding Tms is almost always observed (see reference 4 for an exception). In particular, Franza and Garrels (6) observed that virally transformed rodent REF-52 fibroblasts exhibited reduction in Tm1 and Tm2 of 61 to 99%, Tm4 of 11 to 73%, and Tm6 of 9 to 99%, whereas Tm5 was relatively unmodulated. The

TABLE 5. Ratio[a] of tropomyosin isoforms to total β- and γ-actins in whole cells

Tropomyosin isoform	Parental KD cells	Subcolonial control cells (KDtm-1)	KDsm-5	KDsm-9 passage 4	KDsm-9 passage 11
Tm1	0.040	0.059 (1.47)[b]	0.021 (0.51)[b]	0.042	0.029 (0.69)[c]
Tm6	0.022	0.020 (0.93)	0.005 (0.24)	0.018	0.007 (0.39)
Tm2	0.014	0.017 (1.18)	0.003 (0.22)	0.017	0.007 (0.41)
Tm3	0.024	0.021 (0.87)	0.003 (0.11)	0.021	0.016 (0.76)
Tm4	0.019	0.023 (1.21)	0.009 (0.46)	0.022	0.020 (0.91)
Tm5	0.012	0.016 (1.34)	0.011 (0.94)	0.022	0.021 (0.95)
All Tms	0.131	0.156 (1.19)	0.052 (0.40)	0.142	0.100 (0.70)
Tm1 + Tm2 + Tm3 + Tm6	0.100	0.117 (1.17)	0.032 (0.32)	0.098	0.059 (0.60)
Tm4 + Tm5	0.031	0.039 (1.26)	0.020 (0.65)	0.044	0.041 (0.93)

[a] Values are ratios of the [35S]methionine counts per minute in tropomyosin isoforms and in total β- and γ-actin. All KD strains were labeled for 6 h as described previously (19). The ratio of mutant β-actin to wild-type β- plus γ-actin was 0, 1.03, 0.04, and 1.12 for KDtm-1, KDsm-5, KDsm-9 passage 4, and KDsm-9 passage 11 cells, respectively.

[b] The fraction of the ratio found in parental uncloned KD cells is shown in parentheses.

[c] The fraction of the ratio found in KDsm-9 passage 4 cells is shown in parentheses.

changes in the Tm isoforms in KDsm-5 cells were essentially identical: Tm1 was reduced by 49%, Tm2 by 83%, Tm4 by 54%, and Tm6 by 76%, and only Tm5 was not modulated.

The most highly downregulated Tm in KDsm-5 cells was Tm3, which was reduced by 89%. Franza and Garrels (6) found that the Tm3 response of REF-52 fibroblasts was highly variable depending on the transforming agent and displayed a range from twofold stimulation to a >99% decrease. Human HuT-12 fibroblasts exhibit a two to threefold upregulation of Tm3 after transformation (Table 6) (19), but in HuT-14 fibroblasts which synthesize the mutant β-actin, Tm3 is slightly reduced (19). However, we show here that Tm3 synthesis was downregulated 35% in the primary transfectant strain HuT-12sm4 and 69 to 81% in all tumor-derived strains for transfected HuT-12 cells. Therefore, in this cell system, there seem to be two distinct regulatory changes in Tm3 expression. On one hand, Tm3 upregulation appears to be associated with an immortalization step that can accompany nontumorigenic transformation of human fibroblasts. On the other hand, Tm3 downregulation is associated with further transformation of an immortalized cell to its highly tumorigenic state, as in the tumor-derived transfectant HuT-12 strains and in HuT-14 cells (19). Tm3 downregulation was consistently found in all transfectant strains that synthesized mutant β-actin in excess or in nearly equal amounts of wild-type β- and γ-actin. Thus, Tm3, along with Tm1 and Tm6, appears to be consistently affected by abundant expression of mutant β-actin.

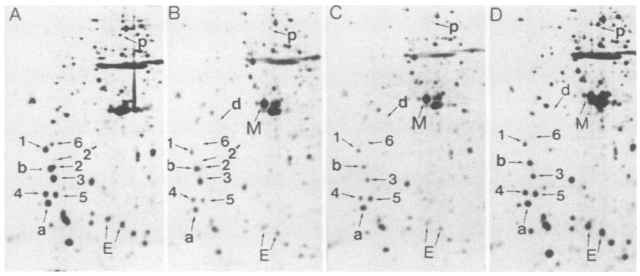

FIG. 5. Autoradiographs of [35S]methionine-labeled polypeptides from parental HuT-12 (A), HuT-12sm11 (B), HuT-12sm11T (C), and HuT-14 (D). The following polypeptides are identified: mutant β-actin (M), tropomyosin isoforms 1 through 6 (numbers 1 to 6) (19), transformation-induced plastin (P) (8, 19), transformation-induced epidermal growth factor-related polypeptides (E) (3, 19), and polypeptides a, b, and d (41 kilodaltons, pI 4.9).

Finally, expression of the mutant β-actin induced the synthesis of a new polypeptide, d, which was also detected in HuT-14 cells but not in KD cells. We conclude that some changes in HuT-14 cells, including its transformed morphology, tropomyosin expression pattern, and induction of a specific marker protein, can be largely accounted for by the mutation in β-actin. Thus, the mutation in β-actin which arose during generation of the transformed HuT-14 cell line is not an epiphenomenon unrelated to other aspects of the HuT-14 phenotype.

It is clear, however, that this mutation cannot account for the complete transformation of HuT-14. In particular, the expression of the mutant β-actin does not influence the growth potential of KD cells (21). This implies that alterations in cell morphology and in Tm expression can be uncoupled from immortalization of human cells. We therefore propose that the mutation in β-actin contributed in a subtle but positive way to the transformation of HuT-14 cells but was insufficient to generate immortalized cells. The finding that HuT-12 cells transfected with the singly-mutant β-actin acquired tumorigenicity implies that this mutation complemented a separate immortalization event to promote expression of the fully tumorigenic state acquired by HuT-14 cells.

Tumor passage selects for increased mutant β-actin. The

TABLE 6. Ratio[a] of tropomyosin isoforms to total β- plus γ-actins in control and transfected HuT-12 cells

Tropomyosin isoform	HuT-12	HuT-12sm2	HuT-12sm2T	HuT-12sm4	HuT-12sm4T1	HuT-12sm8	HuT-12sm8T	HuT-12sm6T	HuT-12sm11T
Tm1	0.026	0.026 (1.00)	0.010 (0.38)	0.020 (0.77)	0.016 (0.62)	0.018 (0.69)	0.011 (0.42)	0.012 (0.45)	0.011 (0.42)
Tm6	0.008	0.002 (0.25)	<0.001 (0.13)	0.002 (0.25)	<0.001 (0.13)	0.008 (1.00)	0.003 (0.38)	0.002 (0.25)	0.001 (0.13)
Tm2	0.014	0.008 (0.57)	0.002 (0.25)	0.008 (0.57)	0.002 (0.14)	0.009 (0.65)	0.012 (0.86)	0.012 (0.86)	0.007 (0.50)
Tm3	0.048	0.041 (0.98)	0.015 (0.31)	0.031 (0.65)	0.011 (0.23)	0.036 (0.75)	0.015 (0.31)	0.009 (0.19)	0.013 (0.20)
Tm4	0.018	0.014 (0.78)	0.004 (0.22)	0.011 (0.64)	0.007 (0.39)	0.015 (0.83)	0.011 (0.61)	0.011 (0.61)	0.010 (0.56)
Tm5	0.017	0.015 (0.88)	0.006 (0.35)	0.011 (0.65)	0.013 (0.76)	0.020 (1.15)	0.019 (1.11)	0.013 (0.76)	0.012 (0.71)
All Tms	0.130	0.112 (0.86)	0.038 (0.29)	0.083 (0.64)	0.050 (0.38)	0.106 (0.82)	0.071 (0.55)	0.059 (0.45)	0.054 (0.42)
Tm1 + Tm2 + Tm3 + Tm6	0.095	0.083 (0.87)	0.028 (0.29)	0.061 (0.64)	0.030 (0.32)	0.071 (0.75)	0.041 (0.43)	0.035 (0.37)	0.032 (0.34)
Tm4 + Tm5	0.035	0.029 (0.83)	0.010 (0.29)	0.022 (0.64)	0.020 (0.57)	0.035 (1.00)	0.030 (0.86)	0.024 (0.69)	0.022 (0.63)

[a] Values are ratios of the [35S]methionine counts per minute in tropomyosin isoforms and in total β- and γ-actin. All HuT strains were labeled for 2 h. The ratio of mutant β-actin to wild-type β- plus γ-actin was 0.31, 0.60, 0.88, 1.95, 0.44, 0.64, 0.62, and 1.06 for strains sm2, sm2T, sm4, sm4T1, sm8, sm8T, sm6T, and sm11T, respectively. The fraction of the ratio found in parental uncloned HuT-12 cells is shown in parentheses.

increased tumorigenicity of neoplastic cell lines after passage through mice appears to select for a subpopulation of cells. In each case studied here the selected cells also showed an increase in the synthesis of mutant β-actin at the expense of endogenous wild-type actin. The increased malignancy and augmented mutant β-actin synthesis appear to be causally linked, and several alternative molecular mechanisms could be responsible for increased β-actin expression in these selected cells, including gene amplification or mutations in regulatory regions involved in transcription or translation.

In HuT-14T cells, the twofold elevation in the rate of mutant β-actin (14) appears to be the result of chromosome 7 duplication, where the β-actin gene maps (27), since HuT-14T cells are trisomic for chromosome 7 (1). We have also found that clones of the mutant β-actin allele appear twice as frequently as clones of the wild-type allele in recombinant DNA libraries derived from HuT-14T cells (17, 22). Thus, augmented mutant β-actin synthesis in tumor-derived transfectants could be due to selection for increased gene copy number.

Tropomyosin expression is regulated by microfilament actin composition. Five of the six Tm isoforms were consistently downregulated in transfected KD and HuT-12 cells and in all tumor-derived transfected HuT-12 fibroblasts in which there was a high rate of expression of stable singly-mutant β-actin. In contrast, a high rate of synthesis of the triply-mutant β-actin had little impact on Tm synthesis. This is a remarkable finding, since it demonstrates that the amounts of mutant β-actin present in a cell can directly influence the synthesis of tropomyosin. Furthermore, it demonstrates that Tm reduction is not related to amounts of mutant β-actin transcripts or to rates of mutant β-actin synthesis. It is notable that the greatest reductions in Tm isoforms occurred among those Tm isoforms that bind most strongly to actin (Tms 1, 2, 3, and 6), whereas the more weakly bound tropomyosins (Tms 4 and 5) were less profoundly affected (19, 25). It has been suggested by others (10, 24, 25) that such a change in Tm isoforms would precipitate a breakdown of the stress fibers. However, the changes in tropomyosin expression in KDsm-5 and KDsm-9 cells do not appear to cause widespread breakdown of stress fibers (21), although they may have contributed to the striking change in cell morphology in other ways (21).

Role of the triply-mutant β-actin. How can the triply-mutant β-actin gene be related to the highly tumorigenic phenotype observed in HuT-14T cells? Since the triply-mutant gene has little if any impact on KD cells, it seems likely that this further mutation did not act to positively alter the cell's properties. However, the failure of this more defective mutant to have any impact in and of itself suggests two possible roles for this further mutation since it in effect behaved as a null allele. First, the single mutant, having contributed to the transformed state in a potentially irreversible way, may have inhibited cell growth to a certain extent. Conversion of the single mutant gene to a triply-mutant "null" allele would remove this inhibition. Second, the generation of a null β-actin allele directly resulted in the reduction in steady-state actin levels in HuT-14T cells (19) and as such produced a potentially more serious actin lesion than the original mutation. Inactivation of a β-actin allele also appears to have occurred in MNNG-HOS osteosarcoma cells accompanying activation of the chromosomally linked *met* oncogene (9). Until it is possible to specifically replace one active β-actin allele with a null allele such as the triple mutant, it will not be possible to test this hypothesis.

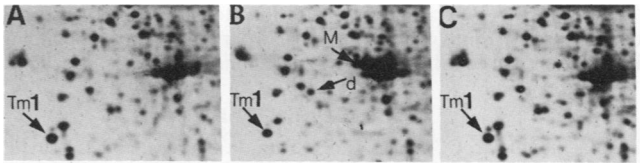

FIG. 6. Long-term autoradiograms (22-day exposures) of [^{35}S]methionine-labeled polypeptides resolved in the 2-D electrophoretic region of polypeptide d (M_r 40,000; pI 4.9) from parental KD cells (A), KDsm-5 (B), and KDtm-1 (C). The following polypeptides are identified: polypeptide d (d), mutant β-actin (M), and Tm1.

Is the mutant β-actin gene an oncogene? NIH 3T3 cells are a partially transformed mouse cell line widely used in defining the oncogenic potential of viruses or DNA segments. These cells are an immortalized line that spontaneously produce foci when grown in culture and rare spontaneous tumors when passaged in nude mice. Human HuT-12 cells are an immortalized cell line with similar characteristics. We show here that high-level expression of transfected copies of the mutant β-actin gene induces tumorigenic conversion of HuT-12 cells. The ability to induce a tumorigenic cell line from nontumorigenic HuT-12 cells by transfection of a defined segment of DNA (in this case the mutant β-actin gene) seems to quality as an oncogenic event. Accordingly, is it fair to suggest that the wild-type β-actin gene is a proto-oncogene? We note with interest that both actin and tropomyosin coding sequences have been found on oncogenes which transform NIH 3T3 cells (23, 26).

That high-level expression of the mutant β-actin gene is not sufficient to induce immortalization or tumorigenesis in all cell lines is obvious from our study of the effects of transfection on normal diploid KD cells (21). Although transfectant strains of diploid KD cells that express abundant levels of mutant β-actin exhibit transformation-sensitive changes in Tm isoform expression similar to those found in HuT-14 and the tumor-derived transfectant strains of this study, the transfected KD cell strains are neither immortalized nor tumorigenic. In addition, the mutant β-actin gene does not induce focus formation in NIH 3T3 cells (unpublished results). However, several well-established oncogenes are incapable of inducing focus formation in NIH 3T3 cells, and alternative tests for oncogenic potential have had to be used (2, 5).

The mutation in β-actin was acquired by HuT-14 cells during mutagenesis which induced the transformed phenotype (18). Although it is clear that actin mutations are not a common occurrence in neoplasia, both qualitative and quantitative modulations in actin and tropomyosin expression and organization are usually found (4, 6, 7, 9, 10, 14, 16, 18–20, 24, 25, 28, 32) following tumorigenic transformation. Together with the ability of mutant β-actin to convert HuT-12 cells into stably tumorigenic lines, the data strongly suggest that reorganization or disruption of the microfilament system plays an important role in the oncogenic process.

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