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Genetics

Identification and order of sequential mutations in β-actin genes isolated from increasingly tumorigenic human fibroblast strains

(actin gene cloning/β-actin expression)

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ABSTRACT **We have sequenced the mutant β-actin gene of a tumorigenic human fibroblast cell line (HuT-14T) and found that it carries three mutations that alter the amino acids at positions 36, 83, and 244 as well as a 22-base-pair "insertion" sequence, in the 5′ intron, not present in a wild-type gene. The less tumorigenic cell line HuT-14, a progenitor of HuT-14T, has the same codon-244 mutation and the insertion sequence but not the other two mutations. A nontumorigenic cell line that is related to HuT-14 but that has no β-actin mutations does carry the intron-length polymorphism. We conclude that the mutation at codon 244 occurred first in a β-actin allele already bearing the 22-base-pair intron insert and that mutations at codons 36 and 83 arose subsequently during the selection for the HuT-14T phenotype. Rat-2 cells synthesize the appropriate charge-variant species of mutant actin protein when transfected with either the singly or the triply mutated β-actin gene.**

Neoplastic transformation of mammalian cells leads to a dramatic change in their shape and cytoarchitecture. Most notable is the change in spatial organization of the actin filament system of the cytoskeleton. Cells lose the ability to assemble cytoskeletal actin cables after neoplastic transformation induced by oncogenic viruses or chemical carcinogens (1, 2) and during tumor development (3).

In diploid human fibroblasts, two electrophoretically distinguishable actin isoforms, β- and γ-actin, polymerize into microfilaments that are a major structural element of the cytoskeleton (2, 4). Because of their contractile properties these microfilaments participate in the processes of cellular motility and cytokinesis. The two isoforms are expressed from separate single genes (5, 6).

Four neoplastic human fibroblast (HuT) strains were induced *in vitro* by treating diploid KD fibroblasts (7, 8) with the chemical carcinogen 4-nitroquinoline-1-oxide (8). One strain synthesized an electrophoretically variant actin (7). The β-actin variant resulted from a mutation at amino acid 244 leading to a glycine-to-aspartic acid exchange (4). Only the HuT strain that contains the mutant β-actin (HuT-14) consistently produced subcutaneous tumors in athymic mice (2, 9). With the exception of the mutant β-actin and the level of tumorigenicity exhibited by HuT-14 cells, this strain exhibited no other characteristics that distinguished it from the three other, less tumorigenic strains (2, 7, 9, 10).

A substrain of HuT-14, HuT-14T, was selected by cloning and tumorigenic growth in athymic mice. The HuT-14T strain exhibits significantly greater tumorigenicity (2), chromosomal rearrangement (11), higher cloning frequency in soft agarose (12), reduced levels of fibronectin in the extracellular matrix (2), and changes in the organization of cytoskeletal actin (2). Each of these phenotypic changes occurred concomitantly with further changes in mutant β-actin biosynthesis (2, 11, 12). As the HuT-14 cells were subcloned, the mutant β-actin of one substrain, HuT-14(6TG^r), exhibited a shift in isoelectric point corresponding to an additional negative charge and became a labile protein with a half-life of 2 hr (2). A subsequent change occurred during the final step that led to the derivation of the more tumorigenic HuT-14T substrain. This final selection event was accompanied by a 2-fold increase in the rates of mutant β-actin protein synthesis and mRNA transcription relative to these rates of coexpression for wild-type β-actin (ref. 2 and unpublished results).

By molecular cloning, we have isolated multiple copies of the human β-actin gene from HuT-14 and HuT-14T cells (6) and additional copies of this gene from HuT-12, a nontumorigenic transformant of KD cells that has no apparent β-actin mutation (6). Mutant copies of the β-actin gene were initially identified by detection of a *Hae* III restriction site polymorphism predicted for the codon-244 mutation (6). We have now determined the complete nucleotide sequence for the mutant gene from HuT-14T. In addition to confirmation of the point mutation at codon 244, we have found two additional structural gene mutations that occurred after the codon-244 mutation. We have also found a 22-base-pair (bp) "insertion" sequence in the 5′ intron, 132 bp downstream from the cap site. This 22-bp sequence is absent from a wild-type gene (13) and from the unmutated allelic gene, allowing a diagnostic assay for the identification of the two alleles from the related but unmutated HuT-12 cell line. Thus, the 22-bp insertion appears to be an intron-length polymorphism present in the original human KD cells.

MATERIALS AND METHODS

All clones of human β-actin genes were isolated and characterized previously (6). DNA sequencing was performed either with M13 subclones, using the method of Sanger *et al.* (14), or with restriction fragments, using the method of Maxam and Gilbert (15). Sequence data was managed by the Intelligenetics GEL program. Polyacrylamide gel electrophoresis of DNA digested with restriction enzymes was described previously (6).

Rat-2 cells (16) were transfected with mutant β-actin genes by coselection for the herpes simplex thymidine kinase (TK) gene (17) or for the neomycin-resistance gene (pSV2neo) using the method of Southern and Berg (18). In each case, 1 μg of plasmid containing the TK gene or pSV2neo was coprecipitated with 10 μg of plasmid containing the β-actin gene. Hypoxanthine/aminopterin/thymidine- or G418 (geneticin)-supplemented culture medium used to select transfectant cell clones was employed for only the initial 6–8 days of selection. Large colonies that grew up in the selective medium were isolated after 12–14 days by trypsinization. At that time, trypsinized cells were inoculated directly into a

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Abbreviation: bp, base pair(s).

Table 1. Nucleotide sequence differences between allelic β-actin-gene clones

Location		Nucleotide(s)			
		Wild-type		Mutant	
Gene segment	Nucleotide number*	M1β1-2	14Tβ17	14Tβ21	14β27
5′ flanking	−84 to −85	Deletion	—	A	—
Intron 1	105	T	—	G	—
	110–111†	No insertion	No insertion	Insertion‡ (22 bp)	Insertion‡ (22 bp)
	368	T	—	Deletion	—
	495	T	—	C	—
	502	T	—	Deletion	—
	556	T	—	C	—
	878	T	—	C	—
Exon 1 (codon 36)	1023,1024†	GG	GG	AA	GG
Exon 2 (codon 83)	1299†	G	G	C	G
Intron 3	1676	T	—	C	—
	1839	C	—	T	—
Exon 3 (codon 244)	2225†	G	G	A	A
Intron 4	2343	G	—	Deletion	—
Intron 5	2592	G	—	Deletion	—
	2608 or 2613	C or G, *Pvu* II site	—	No *Pvu* II site	—
3′ untranslated	2991	A	—	G	—

Clones are described in reference 6. M1β1-2 represents a β-actin gene from a human fetus; 14Tβ17, a wild-type allele from HuT-14T cells; 14Tβ21, a mutant allele from HuT-14T cells; and 14β27, a mutant allele from HuT-14 cells.
*From the β-actin gene sequence of Ng *et al.* (13).
†Difference between the wild-type and mutant alleles of HuT-14T cells.
‡TCACGGCCCGGCCGCAGGCGGC.

15-mm well for metabolic labeling with [^{35}S]methionine. Transfected cells were labeled with [^{35}S]methionine for 4 hr as described (7). Two-dimensional gel electrophoresis of total cellular protein was as described (19).

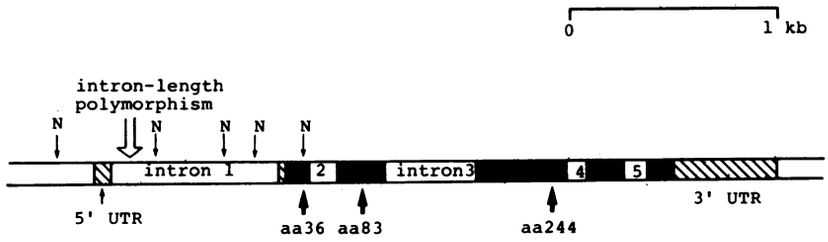
RESULTS

Previously, by testing for the loss of a *Hae* III site at codon 244, we showed that two out of three β-actin-gene clones from HuT-14 cells (14β27 and 14β29) and two out of three β-actin-gene clones from HuT-14T cells (14Tβ21 and 14Tβ24) represented the mutated β-actin gene (6). All of the β-actin-gene clones that have been isolated from each cell source were derived from separate ligation and packaging events (6) and therefore are independent recombinant copies. Prior to these cloning attempts, the wild-type β-actin gene clone had already been isolated from a human fetal genomic library (6). The complete nucleotide sequence of this β-actin gene has been determined (13). We chose to determine the complete nucleotide sequence of one of the mutant-gene clones isolated from HuT-14T cells (14Tβ21) to discover all of the mutational variations that might have occurred between normal KD cells and the most divergent state, manifested by HuT-14T cells. After comparing the differences between the sequence of the mutant allele of HuT-14T and the wild-type gene (derived from a human fetus), we then examined the relevant portions of additional clones of the β-actin genes from various HuT cell lines to establish the order in which the amino acid-altering changes occurred.

A complete catalog of the differences between the wild-type β-actin-gene sequence (13) and the sequences of the mutant genes is presented in Table 1. Three mutations in 14Tβ21 exons all lead to amino acid changes. No other exon differences were found. Twelve additional single-base changes were found in the gene, including one (nucleotide at −83) in the immediate vicinity 5′ of the cap site. Eleven occurred in the introns and one in the 3′ nontranslated region. Four of these additional changes were single-base deletions and the rest were single-base substitutions. We have not determined whether any of these noncoding differences exist as polymorphisms among wild-type genes or whether they arose during the derivation of the tumorigenic cell lines. If these differences do prove to distinguish the wild-type alleles, then they would represent a polymorphism occurring at a frequency of 1%.

Confirmation of the Codon-244 Mutation. We first examined the nucleotide sequences in the region of codon 244 to confirm the predicted nucleotide changes between clones of wild-type and mutant actin gene alleles of HuT-14 and HuT-14T cells. The sequences of the cDNA clone derived from a human fibroblast β-actin mRNA and the functional β-actin-gene clone from a human fetal library both encode glycine at codon 244 (GGC) (13, 20). Furthermore, the nucleotide sequence of another functional β-actin-gene clone (14Tβ17 from HuT-14T cells) was identical in this region. The mutant β-actin gene 14Tβ21 (from HuT-14T cells) (6) was sequenced mostly by the M13 sequencing method of Sanger *et al.* (14). Both strands of the DNA region that spans codon 244 were sequenced. The sequences found confirmed unambiguously the previous conclusion (4, 6) that codon 244 is GAC (Fig. 1) and codes for aspartic acid rather than glycine. The sequences in this region from two additional mutant β-actin-gene clones, 14Tβ24 (from HuT-14T cells) and 14β27 (from HuT-14 cells), were identical to those found in the 14Tβ21 clone (Table 1). These results provide direct proof that a mutation has occurred leading to replacement of glycine with aspartic acid at amino acid 244 in human β-actin (4), that this mutation is present in the original HuT-14 cell line and all subsequently derived cell lines, and that the allelic gene in both HuT-14 and HuT-14T cells does not carry this mutation.

A Charge-Altering Mutation at Codon 36 in a HuT-14T β-Actin Gene. A second mutation that would explain the −2 net change in charge of β-actin in HuT-14T cells was detected in the sequence of the mutant β-actin gene from these cells. Sequencing of both strands of a DNA fragment spanning codon 36 showed unambiguously that codon 36 of the mutant gene clone 14Tβ21 was GAA, which codes for glutamic acid, rather than GGG, which codes for glycine in both the wild-type fetal gene (Fig. 1) (13) and the cDNA sequence of β-actin mRNA (20). This mutation also alters the sequence of a *Nar* I restriction site (from GGCGCC to AACGCC) There



	Intron 1	Gly	Glu	Gly	Verification methods
KD wild-type allele 1	832 bp	GGG	GAG	GGC	I
KD wild-type allele 2	854 bp	GGG	GAG	GGC	II
HuT-14 wild-type allele	832 bp	GGG	GAG	GGC	III
HuT-14 mutant allele	854 bp	GGG	GAG	GAC	IV
HuT-14T wild-type allele	832 bp	GGG	GAG	GGC	V
HuT-14T mutant allele	854 bp	GAA	GAC	GAC	VI
		Glu	Asp	Asp	

FIG. 1. The human β-actin gene and locations of mutations present in HuT-14 cells and derivatives. Scale at upper right represents 1 kilobase (kb). Solid bars represent the five exons and the numbered open bars represent the five introns; hatched bars represent 5′ and 3′ untranslated regions (UTR). Three large upward arrows indicate the three mutations found in HuT-14T cells [at amino acid (aa) codons 36, 83, and 244, respectively]. *Nar* I (N) sites and the location of the 22-bp "insert" are indicated by downward arrows. Below the gene map, differences between wild-type and mutant alleles are listed. Nucleotide changes are underlined. Amino acids specified by the wild-type and the mutant alleles are given above and below the codons, respectively. Verification methods were as follows: (I) Codons are based on sequences of cDNA (20) and the functional gene (13). Intron length is based on the *Nar* I digestion pattern of a clone of HuT-12 wild-type allele 1, which is represented by functional gene clone M1β1-2 (data not shown). (II) Codons are based on sequences of cDNA (20) and the functional gene (13). Intron length is based on *Nar* I digestion pattern of a clone of HuT-12 wild-type allele 2, which is the same as the mutant allele from HuT-14 (data not shown). (III) Codons 36 and 244 are based on *Nar* I and *Hae* III digestion patterns of clone 14β30 DNA (ref. 6 and data not shown). Codon 83 is assumed to be identical in all wild-type genes. Intron length is based on the *Nar* I digestion pattern of clone 14β30 DNA, which is the same as the functional gene clone M1β1-2 (data not shown). (IV) Codons are based on sequences of clone 14β27 and on *Hae* III (6) and *Nar* I (Fig. 2) digestion patterns of clones 14β27 and 14β29. Intron length is based on partial sequencing of clone 14β27 and the *Nar* I digestion pattern of clones 14β27 and 14β29 (Fig. 2). (V) Codon 244 is based on the sequence of clone 14Tβ17 and on the *Hae* III digestion pattern (6). Codon 36 and intron length are based on the *Nar* I digestion pattern of clone 14Tβ17, which is the same as the functional gene clone M1β1-2 (Fig. 2). Codon 83 is assumed to be identical in all wild-type genes. (VI) Codons are based on sequences of clones 14Tβ21 and 14Tβ24 and on *Hae* III (6) and *Nar* I (Fig. 2) digestion patterns. Intron length is based on sequencing of clones 14Tβ21 (complete) and 14Tβ24 (partial) and on the *Nar* I digestion pattern (Fig. 2).

are only four *Nar* I sites in the human β-actin gene, with the other three sites all located in the 5′ intron (intron 1). *Nar* I digestion of the wild-type gene generates a 207-bp DNA fragment with codon 36 at its 3′ end. This fragment would be missing from a gene with a codon-36 mutation. To test this prediction, one wild-type copy of the gene (14Tβ17), two mutant genes from HuT-14T (14Tβ21 and 14Tβ24), and two mutant genes from HuT-14 (14β27 and 14β29) were digested with *Nar* I and the digestion products were compared by electrophoresis in a polyacrylamide gel (Fig. 2). As expected, the wild-type gene (lane G) and the two mutant genes from HuT-14 (lanes C and D) exhibited the 207-bp fragment indicative of the wild-type sequence at codon 36. The two mutant genes from HuT-14T (lanes E and F) lacked the 207-bp fragment.

In addition, the presence of the *Nar* I site in the mutant gene isolated from HuT-14 cells (Fig. 2, lanes C and D) demonstrates that both nucleotides in the GG-to-AA double mutation detected in the HuT-14T cells were generated only after the generation of subclones from the original HuT-14 cells. The wild-type sequence at codon 36 within the HuT-14 mutant gene has been confirmed by sequencing (Table 1).

A Conservative Mutation at Codon 83. A third and unexpected change in the amino acid sequence of β-actin encoded by the HuT-14T mutant gene was detected in codon 83 (Fig. 1). The sequencing of this region was repeated in three independent M13 clones of the HuT-14T gene 14Tβ21, each time demonstrating codon 83 to be GAC, coding for aspartic acid, rather than the normal GAG, coding for glutamic acid, as found in the wild-type gene (13) and the cDNA sequence of the mRNA (20). A DNA fragment spanning codon 83 (Fig. 1) was then isolated from a mutant gene from HuT-14 cells (14β27) and the other mutant gene from HuT-14T cells (14Tβ24). Sequencing of these fragments showed that this codon-83 mutation, although present in both copies of the mutant gene of HuT-14T, was absent from the mutant gene of HuT-14 cells (Table 1). Therefore, this third structural-gene mutation must have occurred either at the same time as the codon-36 mutation or at a later stage in the derivation pathway of the HuT-14T substrain.

An Intron-Length Polymorphism Within the 5′ Intron. Comparison of the *Nar* I digestion patterns shown in Fig. 2 revealed one additional difference that distinguished all mutant β-actin genes from the wild-type genes of HuT-14 and HuT-14T. A DNA fragment generated by *Nar* I digestion of a wild-type gene migrated just ahead of the 434-bp marker in lane G and appeared to be replaced in lanes C through F by a slightly larger *Nar* I fragment that migrated slightly slower than the 434-bp marker. This fragment is 430 bp long in the wild-type β-actin gene sequence (13). We sequenced this region from the wild-type gene 14Tβ17 and found the intron sequence to be identical to that of the fetal gene (Table 1) (13). In contrast, the same fragment from the three mutant genes 14β27, 14Tβ21, and 14Tβ24 contained a new 22-bp sequence (Fig. 1, Table 1) inserted in the first intron, 110 bp downstream from the transcriptional start signal.

We then tested 12 independent clones of the β-actin gene derived from three different cell lines for the presence of this "insertion" sequence, using this *Nar* I fragment size polymorphism as the indicator of the insertion sequence. Two out of three clones of the β-actin gene from HuT-14 and five out of seven isolated from HuT-14T exhibited the insertion sequence demonstrated by the *Nar* I assay (data not shown). The insertion sequence was found only in those clones from

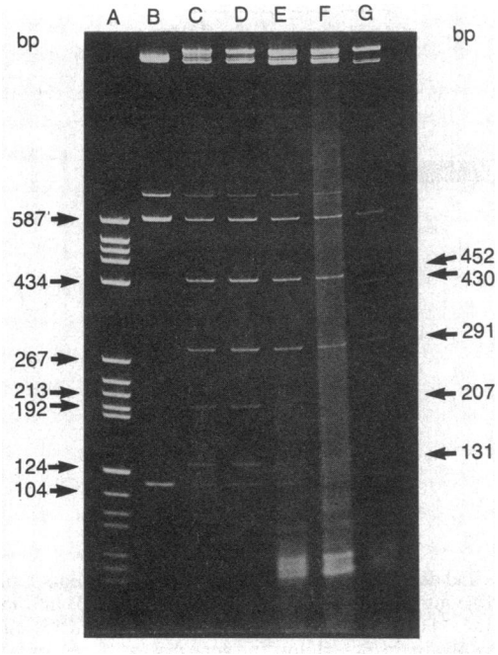


FIG. 2. Ethidium bromide-stained 5% polyacrylamide gel after electrophoresis of *Nar* I restriction fragments of plasmids produced by insertion of different β-actin genes into pBR322. Lanes: A, *Hae* III-digested pBR322 (markers); B, *Nar* I-digested pBR322; C, *Nar* I-digested p14β29; D, *Nar* I-digested p14β27; E, *Nar* I-digested p14Tβ24; F, *Nar* I-digested p14Tβ21; G, *Nar* I-digested p14Tβ17. p14β27 and p14β29 are separate clones of the mutant β-actin gene from HuT-14 cells, p14Tβ24 and p14Tβ21 are separate clones of the mutant β-actin gene from HuT-14T cells, and p14Tβ17 is a clone of the wild-type β-actin gene from HuT-14T cells (6). The *Nar* I digestion pattern of p14Tβ17 is identical to that of pM1β1-2 (not shown).

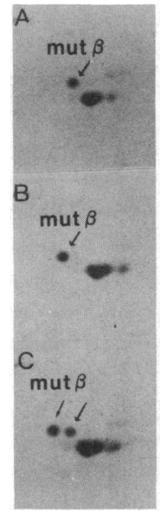


FIG. 3. Two-dimensional polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled actin polypeptides in total protein of transfected Rat-2 cells (16); only relevant portions of the autoradiographs are shown. (*A*) Hypoxanthine/aminopterin/thymidine-resistant cell line cotransfected with the mutant β-actin-gene clone p14β29 and the thymidine kinase gene in pHSV106 (17). (*B*) Antibiotic G418-resistant cell line cotransfected with the mutant β-actin-gene clone p14Tβ24 and pSV2neo (18). (*C*) Superimposition of *A* and *B*. The more acidic mutant β-actin species on the left in *C* came from *B*.

HuT-14T that were missing the *Nar* I site at codon 36. Furthermore, *Nar* I digests of one of two additional clones from the HuT-12 cell line (7), which lacks the mutations at codons 36 and 244, also displayed a size polymorphism in this *Nar* I fragment (data not shown).

Expression of Mutant Genes After Gene Transfer. For final verification that the cloned mutant genes from the HuT-14 and HuT-14T cell lines are responsible for synthesis of the two charge-variant actin polypeptides, we transfected these cloned genes into thymidine kinase-deficient Rat-2 fibroblasts, using coselection for the herpes simplex type 1 thymidine kinase gene (17) or the pSV2neo gene, which confers resistance to antibiotic G418 (18). Thirty-two percent (11 out of 34) of the hypoxanthine/aminopterin/thymidine- or G418-resistant colonies that arose after transfection expressed the exogenous mutant β-actin protein. Cells transfected with the mutant gene isolated from the HuT-14 cell line (14β29) (Fig. 2) synthesized a protein that migrated to the same position in two-dimensional electrophoresis as the variant β-actin expressed in the HuT-14 cell line (Fig. 3*A*). The difference between the apparent isoelectric point of this variant actin and that of wild-type β-actin corresponded to one unit charge per molecule, and the mutant protein was slightly reduced in migration rate in the NaDodSO$_4$/PAGE dimension (4, 7). Cells transfected with the mutant gene isolated from the HuT-14T substrain synthesized a protein that appeared identical to the variant β-actin expressed in the HuT-14T cell line (Fig. 3*B*); this variant polypeptide differed from wild-type β-actin by two unit charges per molecule, based on isoelectric point (2). Overlaying of the two-dimensional gel patterns from the two transfectant cell types (Fig. 3*C*) shows that wild-type actins and other common proteins are superimposed, thus confirming that the two exogenous mutant human genes produce variant polypeptides with the charge relationship expected from the sequential replacement of two neutral amino acids by two acidic amino acids.

DISCUSSION

Nucleotide sequencing of the mutant human β-actin gene from the HuT-14T cell line has established that three mutations occurred in one of the two functional allelic β-actin genes of KD cells. These mutations all occurred in a gene bearing a 22-bp intron-1 length polymorphism which appears to represent a polymorphic locus among human β-actin genes. The first mutation, at codon 244, occurred during induction of the transformed state of the HuT-14 strain after treatment of KD cells with the mutagen 4-nitroquinoline-1-oxide (7). The concomitant occurrence of this point mutation and induction of neoplastic transformation is suggested by the presence of a mutant β-actin in the HuT-14.2 cell line (7), which was derived from a different KD cell focus. This additional transformed focus appeared in a separate subculture of the same mutagenized KD cell stock that produced the HuT-14 focus (7). Thus, presumably, HuT-14 and HuT-14.2 arose from a common stem cell shortly after mutagenesis (7).

A second mutation, at codon 36, may have occurred at the time of subcloning of the HuT-14 cell line in 6-thioguanine-supplemented medium (2). However, we think that it is more likely that this second mutation occurred spontaneously during long-term cultivation of the HuT-14 cell line before the subcloning step was performed. Examination of high-resolution profiles obtained by two-dimensional gel electrophoresis of proteins from the original highly cultivated HuT-14 cells reveals a minor polypeptide species (p153) in the electrophoretic position of the doubly charge-altered mutant β-actin (10).

The third mutation at codon 83 also occurred after the codon-244 mutation, since the codon-83 mutation was not found in the mutant β-actin gene (14β27) of HuT-14 cells. Whether the mutation at codon 83 was coincident with that at codon 36 is unclear. If we had cloned and sequenced the

mutant β-actin gene from the intermediate subclonal cells, HuT-14-6TG^{r3} and HuT-14UVcl1, that were produced before the tumorigenesis step that produced the HuT-14T substrain (2), then we might have been able to establish the exact order of occurrence of these three mutations. We have chosen instead to study these mutations with genetically engineered β-actin genes carrying the mutations independently. This approach has been taken because the phenotypic consequence of each of these mutations can only be established by reconstruction experiments in which a gene with each mutation or pair of mutations is reintroduced into KD cells and other human diploid fibroblast strains or into other neoplastic strains such as HuT-12 (which is similar to HuT-14 but lacks evidence of actin mutations). Such reconstruction experiments should ultimately establish the phenotypic changes that arise from the three distinct β-actin mutations that we have defined here.

Why did frequent sequential mutations occur in only one of the two β-actin alleles in the KD/HuT-14 cell lineage? Might the subsequent mutations provide a selective growth advantage to counteract the effect of the first mutation? Although the HuT-14 cell line is highly tumorigenic, its mutant β-actin with the single amino acid change at residue 244 is a stable protein that is incorporated into the cytoskeleton (2). The presence of this defective actin in the cytoskeleton may have a subtle inhibitory effect on the proliferation of the HuT-14 cell line. Subpopulations of cells might arise that have spontaneously incurred additional mutations that increase the lability of the mutant β-actin protein, diminishing its incorporation into the cytoskeleton (2). Thus the latter mutations might ameliorate the effect of the original mutation by engendering a more defective but more labile gene product.

The final genetic change that occurred in the generation of HuT-14T cells does not necessarily support this hypothesis. This change led to an ≈2-fold increase in the rate of synthesis of triple-mutant β-actin RNA transcripts and polypeptide products (2). Simultaneously, a cell line was produced that exhibited greater tumorigenicity (2). The ratio at which mutant to wild-type genes were isolated from the HuT-14T cell (5:2) is consistent with the prediction that the mutant β-actin gene had duplicated (2). This explanation now appears to be correct since human chromosome 7, known to carry the β-actin gene (13), has become trisomic in HuT-14T cells (11).

How would β-actin mutations at amino acids 36, 83, and 244 govern phenotypic changes in cells? One obvious possibility is that microfilaments that contain mutant β-actin may have abnormal conformations. Consequently, all microfilament functions could be affected to various degrees by the presence of mutant β-actin. El-Saleh *et al.* (21) have shown that lysine-237 of both G- and F-actin can be modified chemically without affecting microfilament polymerization and depolymerization and that such modification can alter the conformational interaction between actin and tropomyosin. Consistent modulations of tropomyosin and actin expression accompany transformation of rat fibroblasts and thus are implicated in manifestation of the neoplastic state (22, 23). Chemical-modification studies have also implicated histidine-40 in actin polymerization (24). Mutations near these sites might also affect actin–actin interactions as well as interactions between actin and microfilament-binding proteins which in turn may affect cellular phenotype. Actin has been found associated with transcriptionally active chromatin (25) and has been reported to be a component of the reconstituted transcriptional complex (26). Thus, mutations in β-actin conceivably could affect gene expression by altering transcriptional activity.

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