Journal of Cellular Biochemistry 25S:197–204 (1996)

G-Actin as a Risk Factor and Modulatable Endpoint for Cancer Chemoprevention Trials

George P. Hemstreet, III,[1,2,3,4,6]* JianYu Rao,[1,8] Robert E. Hurst,[1,2,5] Rebecca B. Bonner,[1] Przemko Waliszewski,[1] H. Barton Grossman,[7] Monica Liebert,[7] and Barbara L. Bane[2,6]

[1]Department of Urology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma
[2]Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma
[3]Department of Occupational and Environmental Health, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma
[4]Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma
[5]Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma
[6]Department of Veterans Affairs Medical Center, Oklahoma City, Oklahoma
[7]Department of Urology, University of Texas M.D. Anderson Cancer Center, Houston, Texas
[8]Department of Pathology, University of California, Los Angeles, California

Abstract Because tumorigenesis is an ongoing process, biomarkers can be used to identify individuals at risk for bladder cancer, and treatment of those at risk to prevent or slow further progression could be an effective means of cancer control given accurate individual risk assessment. Tumorigenesis proceeds through a series of defined phenotypic changes, including those in genetically altered cells destined to become cancer as well as in surrounding normal cells responding to the altered cytokine environment. A panel of biomarkers for the changes can provide a useful system for individual risk assessment in cancer patients and in individuals exposed to carcinogens. The use of such markers can increase the specificity of chemoprevention trials by targeting therapy to patients likely to respond, and thereby markedly reduce the costs of the trials.

Previous studies in our laboratories showed the cytoskeletal proteins G- and F-actin reflect differentiation-related changes in cells undergoing tumorigenesis and in adjacent "field" cells, and a pattern of low F-actin and high G-actin is indicative of increased risk. Actin changes may be a common feature in genetic and epigenetic carcinogenic mechanisms. In a group of over 1600 workers exposed to benzidine, G-actin correlated with exposure, establishing it as an early marker of effect. In another study, a profile of biomarkers was monitored in patients who underwent transurethral resection of bladder tumor (TURBT) and received Bacillus Calmette Guerin (BCG) and/or DMSO. The primary objective was to determine how the defined biomarkers expressed in the tumor and the field correlate with clinical response and recurrence. DMSO, known to modulate G-actin in vitro, was used as an agent. Results strongly support the hypothesis that cytosolic G-actin levels measured by quantitative fluorescence image analysis (QFIA) can be an important intermediate endpoint marker for chemoprevention and that the p300 (M344) and DNA ploidy markers identify a high-risk group that requires more aggressive therapy and recurrence monitoring. Further research with other markers has shown that DD23 and nuclear actin, both of which identify late, specific changes, may increase the battery of useful markers. Taken together these studies show how biomarkers are employed to study individuals at risk, aid in the selection of chemopreventive compounds and assist in the understanding of the pathogenesis of malignancy. J. Cell. Biochem. 25S:197–204. © 1997 Wiley-Liss, Inc.

Key words: biomarkers; chemoprevention; cancer risk factor; G-actin; retinoids

Recent developments in the study of intermediate endpoint markers establish the rationale for assessing individual risk for premalignant disease, designing strategies for cancer prevention, and understanding the fundamental mechanisms of carcinogenesis. [1–4] Advances in molecular biology, epidemiology and the human genome project provide the tools and

Contract grant sponsor: NIH, contract grant number 5-U01-CA56981, contract grant number 5-RO1-OH 02647, contract grant number R01-DK45368; Contract grant sponsor; U.S. Department of Veterans' Affairs, contract grant number: Merit Review 111-34-0732.

*Correspondence to: Dr. George P. Hemstreet, Department of Urology, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73190.

Received 12 December 1995; Accepted 8 April 1996

© 1997 Wiley-Liss, Inc.

knowledge base for defining and identifying important biomarkers of genetic susceptibility. Complete knowledge bases of markers of susceptibility and markers of exposure theoretically would be adequate for individual risk assessment. The complexities of genetic polymorphism and the inability to precisely quantitate exposure and individual thresholds complicate this strategy. An alternative approach is to evaluate qualitative and quantitative changes in biomarkers of effect with a knowledge of their functional role and when they are expressed in the sequence of events in carcinogenesis.

Based on the original concepts of Ploem [5] and West [6,7], our laboratory developed a technique of quantitative fluorescence image analysis (QFIA) which is used to quantitate the functional molecules important in carcinogenesis at the single cell level with a specific focus on the biologically active proteins. Our focus on the protein molecules is predicated on several important theoretical concepts which recognize that genes may be present but not transcribed and their mRNA may not be translated thus quantitation of DNA or RNA may not necessarily reflect the actual biologically active protein component.

Given the large number of proteins which are functionally modulated in the cascade of events in carcinogenesis, the selection of candidate biomarkers is not a trivial issue. Selected biomarkers should not only show statistically significant alterations but should have the power to impact clinical decision criteria. Thus, selection of biomarkers requires a systematic approach to enhance the current predictors of stage and grade.

Conventionally, the pathologist and cytologist have relied primarily on morphometric features to classify disease risk such as relative degrees of dysplasia. High-grade dysplasia is more likely to be associated with a premalignant state but because it is expressed late in the process of carcinogenesis is less amenable to modulation by chemopreventive compounds, while earlier specific differentiation alterations are potentially susceptible to modulation by less toxic chemoprevention strategies [3]. A particular problem is that early alterations most likely to be susceptible to chemopreventive modulation may not express morphologic changes, thus eliminating conventional pathology as one of the major tools available to identify individuals at risk. Even with enhancement of quantitative morphometric analysis, such changes may not be detected reliably because they represent indirect changes at the cellular structural level while carcinogenesis affects specific molecules. In a study of field disease in the bladder, clear differences in molecular content of biomarkers were seen in cells in the absence of any discernible morphologic changes [2].

Our approach has been that quantitative changes in specific biomarkers more directly reflect the specific alterations that accompany the process of carcinogenesis than does morphology. Quantitation of DNA [8–10], cytoskeletal proteins [2,11], oncogene proteins [2,12–14] and tumor-associated antigens (unpublished results) [15] has elucidated a sequence of biological changes that accompany bladder tumorigenesis. The distributions of specific molecules may be more informative than morphology, which often reflects artifacts of preparation. Neural networks have proven useful in interpreting the distribution of the M344 antigen in bladder cells, distinguishing certain false-positive patterns from those associated with tumorigenesis [16].

F- AND G-ACTIN AS A DIFFERENTIATION MARKER

Several years ago our research group demonstrated that quantitative alterations in F-actin were associated with cellular differentiation and that cells dedifferentiated with phorbol ester could be redifferentiated with DMSO and retinoids with a concomitant switch from low amounts of F-actin in dedifferentiated cells and high amounts in differentiated cells [3,11]. Evidence of the significance of the alterations in bladder cancer was confirmed by the quantitative decrease in F-actin in patients with bladder cancer [17]. The degree of abnormality was associated with the relative degree of risk in a cross-sectional study of patients stratified by bladder cancer risk, which suggested that alterations in actin were a relatively early event in tumorigenesis. More recently, biomarker alterations were studied in the bladder cancer field, the area adjacent to the tumor, and the tumor itself [2]. A hierarchy of G-actin elevation was observed in the tumor compared to the adjacent area and the distant field, confirming a graded distribution and a reciprocal relationship with F-actin expression. These observations of alterations in G-actin associated with biochemical field disease provided strong additional data

that G-actin is an early differentiation marker for risk assessment.

In a recent study of 27 prostate cancer patients, G-actin was positive in the cancer lobe compared to a non-cancer lobe, with a statistical significance of $P = 0.0006$, as shown in Table I. A weak association of altered G-actin with aneuploidy was observed ($P = 0.086$). A correlation was observed with tumor stage ($P = 0.04$). There was no correlation with tumor volume, Gleason score, or grade. These results in many respects parallel the observations in bladder cancer that there is field disease effect and the alterations need not be present in the cancer cells themselves. This is an important observation because it makes it theoretically possible to detect premalignant changes while targeting the precise cells that undergo the final steps in tumorigenesis if the biomarker is an early field alteration. The samples in this study were from patients with biologically active cancer; the biomarker expression in glands with occult disease remains to be defined.

ABNORMAL G-ACTIN AS A RISK FACTOR FOR BLADDER CANCER

G-actin has also been studied as an early marker for risk assessment in a cohort of Chinese workers at risk for bladder cancer following benzidine exposure. The Chinese cohort consisted of some 2,000 exposed workers and 400 controls. In the study, a profile of three biomarkers, an early marker, G-actin, an intermediate marker, M-344 (a tumor-related antigen), and a late marker, DNA 5CER (percentage of cells with > 5C DNA), was evaluated.

TABLE I. Distribution of G-Actin Results by Presence or Absence of Detectable Cancer in Lobe

	G-actin positive (mean >90 units/cell)	G-actin negative (mean ≤90 units/cell)
Cancer detected in lobe	22	6
Cancer not detected in lobe	6	14
	$p = 6.0 \times 10^{-4}$	

Cells were obtained by fine-needle aspiration and labeled for G-actin with fluorochrome-labeled DNaseI. G-actin was measured in approximately 100 unselected individual, single cells by QFIA standardized against a standard cell line. Results are expressed as the mean of the population measured in G-actin units.

After three years of follow-up, nine new cancer cases have been detected. Table II summarizes the findings with a subset of the workers from two of the cities. Importantly, G-actin positivity was associated with a strong correlation with benzidine exposure history and with smoking and BPH, both of which are risk factors for bladder cancer. As a diagnostic test for the presence of cancer, G-actin had a high false positive rate, as would be expected of a marker that was altered early and progresses at different rates in different individuals. Modulation of this biomarker could be used as a surrogate endpoint for chemoprevention in such a cohort if chemoprevention were targeted at the subset of exposed individuals with altered G-actin. G-actin may well serve as a surrogate or intermediate endpoint marker for chemoprevention studies.

G-ACTIN AS A POTENTIAL GUIDE FOR MONITORING CHEMOPREVENTION

Recent data in our laboratory indicate that modulation of G-actin can be used as an intermediate marker for chemoprevention studies. A profile of biomarkers was monitored in a series of patients undergoing transurethral resection of bladder tumor (TURBT), receiving Bacillus Calmette Guerin (BCG) and DMSO. The primary objective of this study was to monitor biomarker expression following surgical excision of the tumor by TURBT followed by intravesical BCG and/or DMSO, depending on the presence of residual disease following resection. Patients determined to be tumor-free (negative cytology and cystoscope examination), were treated with intravesical DMSO once per week for four weeks. DMSO, an FDA-approved drug, was used as to test biomarker modulation because it is known to induce differ-

TABLE II. Significance (p) of Risk Factors in Producing Positive Test Results in Chinese Workers in Two Cities by Test Using Cox Logistic Regression Model

	p Value		
Feature	G-Actin 92/612	DNA 58/626	M344 22/626
Pack Years	.0001	.0001	.0001
Years of Exposure	.0001	.0001	.0001
Prostatic Hyperplasia	.0469	.0176	.0002
Hematuria	.3215	.4558	.6964

Numbers under each marker give the number of positive results and the total number of satisfactory test results.

entiation of cells in vitro and to modulate F- and G-actins as an indicator of response [2,11,17]. The biomarker profile included in this study was derived from the previous field mapping study [2], and consisted of G-actin, a differentiation and field disease marker, M344, a bladder tumor specific antigen, and DNA, a genetic instability marker. G-actin is a very early biomarker for altered differentiation, the M344 antigen detects low-grade tumors and developing dysplasias, and aberrant DNA ploidy, as measured by the percent of cells exceeding 5C DNA, measures the presence of DNA instability that leads to high-grade tumors [2,15]. Bladder wash samples were collected at each visit and the samples were used to monitor DNA, M344 and G-actin expression. Twenty-six bladder cancer patients were studied; 20 of them received six weeks of intravesical BCG, and 14 were treated weekly for four weeks with intravesical DMSO. G-actin, DNA aneuploidy and M344 tumor antigen were evaluated by quantitative fluorescence image analysis (QFIA) on uroepithelial cells from bladder wash samples. Patients were followed for one year, or until a recurrence was detected by cystoscopy. Patients with recurrence were treated and monitoring was continued because the intent of the study was to assess the effect of modulation of biomarkers on recurrence. As shown in Table III, BCG normalized DNA ploidy in 43% of the cases ($P < 0.05$ by Fisher Exact test), while M344 antigen, an intermediate marker, was normalized in 58% of the cases ($P < 0.05$ by Fisher Exact test). G-actin, an early differentiation biomarker, was corrected in only 25% of the cases ($P > 0.05$). DMSO eliminated 43% of abnormal G-actin markers mainly in patients who were only G-actin-positive. Of interest was the observation that 61% of G-actin-negative patients became positive after BCG. These patients also usually remained positive for one or both of the other two markers and recurred early. It is possible these patients have a clone of BCG-resistand cells that rapidly progress to malignancy after the suppressive effect of surrounding normal uroepithelium is eliminated. These results support the hypothesis that the two agents act at different points, with BCG killing dysplastic and cancerous cells while DMSO induces differentiation only in cells from biochemical field disease that are not irreversibly transformed. Excluding patients who did not respond to BCG (and who had repeatedly recurrence correlated with persistent biochemical field disease as defined by abnormal G-actin findings. Although persistence of abnormal DNA and M344 markers were predictors of recurrence, a positive G-actin after therapy was the single most powerful predictor of recurrence. Of 18 patients who were G-actin-positive after therapy, regardless of whether therapy consisted of TUR alone, TUR plus BCG, or TUR plus BCG plus DMSO, 12 (67%) recurred. Of the 15 patients who were G-actin-negative after therapy, only 2 (13%) recurred ($p = 0.015$ by χ^2). These data strongly support the hypothesis that cytosolic G-actin levels measured by QFIA can be an important intermediate endpoint marker for chemoprevention and that normalization of this marker is indicative of a positive chemopreventive response.

TABLE III. Biomarker Expression in Response to BCG and DMSO Therapy of Bladder Cancer

Marker prior to therapy	Marker after therapy	G-actin	DNA (% Cells > 5C)	M344 Antigen
Effect of BCG Therapy				
Positive		8	7	12
	Positive	6 (75%)	4 (57%)	5 (42%)
	Negative	2 (25%)	3 (43%)	7 (58%)
Negative		18	18	13
	Negative	7 (39%)	17 (94%)	11 (85%)
	Positive	11 (61%)	1 (6%)	2 (15%)
Effect of DMSO Therapy				
Positive		7	1	5
	Positive	4 (57%)	0 (0%)	4 (80%)
	Negative	3 (43%)	1 (100%)	1 (20%)
Negative		7	13	9
	Negative	6 (86%)	13 (93%)	5 (56%)
	Positive	1 (14%)	1 (7%)	4 (44%)

The conclusions drawn from those studies are: 1) Abnormal G-actin in the bladder cancer field is a risk factor for bladder cancer recurrence. 2) BCG normalizes biomarker expression late in carcinogenesis, but has little effect on the early marker, G-actin. 3) When DMSO normalizes G-actin, recurrences were reduced, but DMSO does not differentiate cells that have irreversibly progressed. 4) G-actin may be useful as an intermediate endpoint marker for monitoring the effectiveness of chemoprevention therapy.

ACTIN BIOMARKERS IN THE NUCLEUS

Coffey has proposed that DNA organization in the nucleus is an important epigenetic factor in cancer development [18] while others have observed that cell-cell interactions such as gap junction and adherence are also involved [19]. Other experiments studying the relationship between basement membrane and type IV procollagen mRNA expression support a direct interaction among extracellular matrix/cytoskeleton/nuclear matrix [20]. Additional studies show a connection between cytoskeleton actin, the nucleus, and the cell periphery that is altered following transformation [21]. Qualitative alteration of cytoplasmic actin, the major cellular protein involved in cell-cell interaction, morphology, motility, differentiation and adhesion [22–24], has been widely investigated in cancer cells. Quantitative alteration of actin in the cytoplasm, i.e., decreased F-actin and increased G-actin, is related to cellular dedifferentiation which reflects either epigenetic mechanisms, such as effects of tumor promoters [11], stress [25–27], growth factors [28,29], or genetic factors including genetic predisposition [30] and oncogene activity [31–34]. Recently, nuclear actin has been identified as an important nuclear protein involved in DNA-crosslinking [35], transcription control [36,37], cell cycle regulation [38] and chromosome morphology [39,40]. Because most of these nuclear functions are altered in cancer cells, and because profound changes in nuclear structure and function are seen in transformed cells, it seems plausible that a quantitative alteration of nuclear actin might also occur.

The objective of the current study was to investigate quantitative changes in both nuclear and cytoplasmic G- and F-actin utilizing an established in vitro cellular transformation model [41]. Human uroepithelial cell lines, HUC-BC and HUC-PC cells, were immortalized by infection with SV-40 virus and used as the in-vitro model. Although SV-40 large T-antigen is known to bind to and inactivate p53 and Rb, neither line is tumorigenic. The BC and PC lines have similar morphological and biochemical features, but the PC line can be induced to become tumorigenic by treatment with 4-ABP[3] [42] or transfection with mutant *ras* oncogene [43] while the BC line does not become tumorigenic under these conditions and thus serves as a control to identify effects in the HUC-PC cells that are specific for tumorigenesis. F- and G-actin contents were quantified by fluorescence image analysis [2]. Parallel biochemical actin analyses were performed on the cell lysates both to demonstrate that fluorescent probe-binding methods are accurate and to calibrate the QFIA methods in absolute actin content. The molecular structure and characteristics of cytoplasmic and nuclear actin are similar, but not identical [44] but can still be quantified by the similar molecular probes [39]. This study confirmed the hypothesis that nuclear actin is altered in response to 4-ABP exposure. Because only the PC cells, cells with constitutively decreased cytoplasmic actin, can be transformed by the carcinogen 4-ABP exposure and only the carcinogen-treated PC cells showed quantitative alteration of nuclear actin, we hypothesize that a pathway from extracellular matrix/cytoplasmic actin/nuclear actin is important in maintaining the homeostasis of a cell while alterations of the pathway are related to specific events in carcinogenesis.

Table IV shows changes in the nuclear actin in both cell lines in response to 4-ABP. Before exposure to 4-ABP, the PC cells had lower (approximately 1.5-2-fold, $P < 0.01$) cytoplasmic F-actin content, and higher cytoplasmic G-actin content, but similar levels of nuclear G-

TABLE IV. G- and F-Actin Levels in Transformable HUC-PC and Untransformable HUC-BC in Response to Transformation by 4-Aminobiphenyl (4-ABP)

Cell line	Treatment	G-Actin	F-Actin	Total actin
HUC-BC	Control	21 ± 1	38 ± 7	59 ± 8
	4-ABP	20 ± 1	34 ± 4	54 ± 5
HUC-PC	Control	19 ± 2	32 ± 4	51 ± 6
	4-ABP	28 ± 2**	13 ± 2*	41 ± 4

*P < 0.05 in comparison with control, **P < 0.01 in comparison with control.

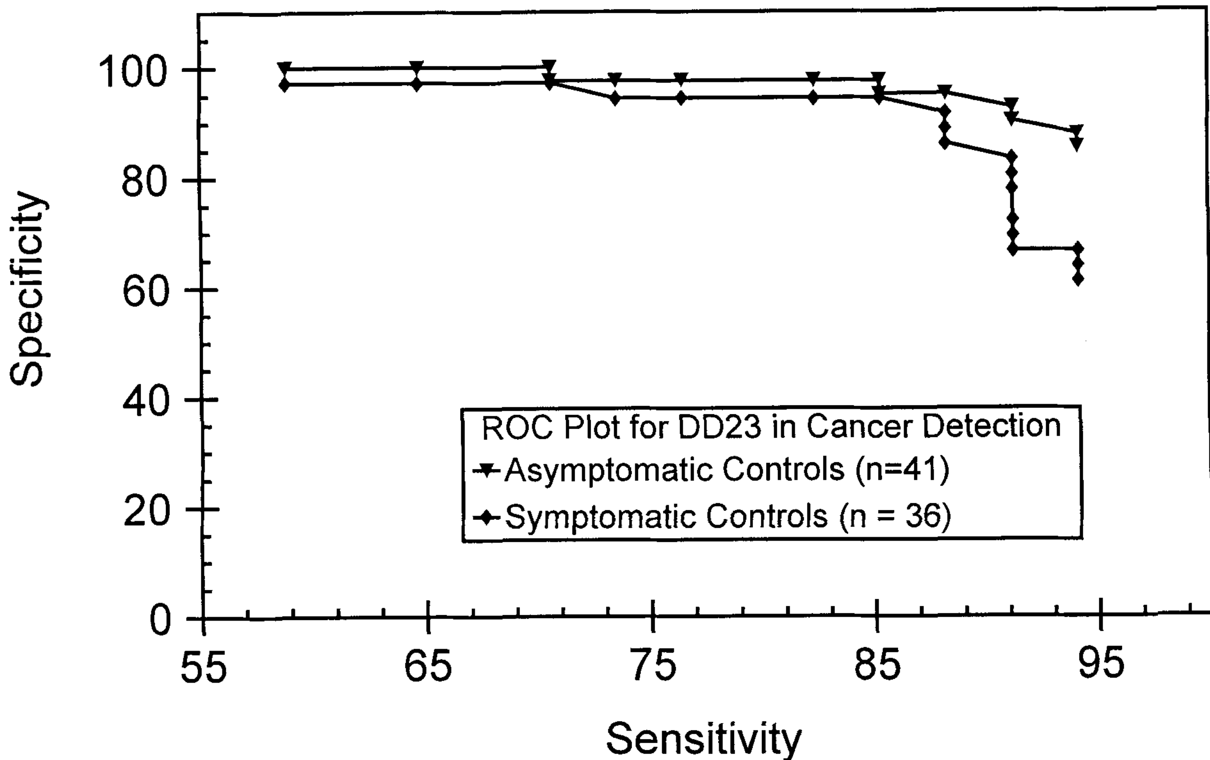

Figure 1. Receiver operating characteristic (ROC) plot of sensitivity as a function of specificity. Sensitivity, the fraction of patients with cancer having positive DD23 results, is plotted against specificity, the fraction of controls having negative DD23 results, at different thresholds to separate positive and negative. The similarity of the plots for the two control populations indicates there are a limited number of clinical urologic conditions causing a false positive DD23 test, thereby enhancing the utility of this test in symptomatic patients.

and F-actin, in comparison with the BC cells. After transformation by 4-ABP, the PC cells showed decreased F-actin and increased G-actin in both cytoplasm and nucleus (about 2-fold in each case, $P < 0.01$) and loss of cytoplasmic F-actin fibers, but only cytoplasmic actin was altered in the BC cells (20%, $0.05 > P > 0.01$). The total nuclear actin content was not altered; only the equilibrium between G- and F-actin seems to have been shifted. Northern blot analysis showed the expression of the b-actin gene was only about 20% lower in 4-ABP treated PC cells than untreated controls, indicating the cellular change in actin was attributed to a shift between F- and G-actin proteins rather than to net actin synthesis. Nuclear actin change was observed only in the transformable PC cells, while the cytoplasmic actin was altered in both cell lines by carcinogen treatment. This study suggests that alteration in nuclear G/F-actin content relates specifically to carcinogen-induced cellular transformation rather than differentiation-related phenomena and suggests a link between the genetic and epigenetic mechanisms of carcinogenesis.

OTHER LATE MARKERS IN CANCER TUMORIGENESIS

The reported results suggest that nuclear actin may be another marker expressed later in the process of bladder cancer tumorigenesis and may be associated with the transformational event. In previous studies we have demonstrated that DNA 5CER is also a late marker but, unfortunately, also has a high false positive rate in cigarette smokers. Our laboratory has investigated a marker, the DD23 antigen, using an antibody developed by Liebert and Grossman [45].

The DD23 monoclonal antibody for the detection of bladder cancer in exfoliated cells has been characterized. The optimal sensitivity and specificity of 85% and 95%, respectively, for low-and high-grade urothelial carcinomas, and including several low-grade Ta/T1 tumors, were determined from the ROC plot shown in Figure

1. This figure shows the sensitivity plotted against the specificity determined with the asymptomatic and symptomatic controls. Among 41 asymptomatic controls, only 2 were positive (5%) and even among symptomatic controls without bladder cancer, a group that has a higher false positive rate for other biomarkers, only 2/36 (6%) were positive for DD23. Among patients being monitored for recurrence without detectable bladder cancer, 3/23 (13%) were positive. This high specificity, even among patients with a previous history of bladder cancer, is unusual. The finding that the marker is expressed in cells that are not apparently cytologically aberrant and that it is expressed in bladder cells in response to cancers at other sites (e.g. prostate, renal cell carcinoma, and a squamous cell carcinoma metastatic to the bladder) supports the possibility that this marker represents a response of the bladder epithelium to signals from transformed cells rather than being a marker only expressed by cells undergoing tumorigenesis. This hypothesis is reinforced by the finding of positive cells well in excess of the few percent of tumor cells identified with molecular genetic markers typically found in urine [46].

SUMMARY

The cytoskeleton represents an early response to the process of carcinogenesis and can act as a marker for its presence. G-actin, the monomeric form of actin, appears to exist in equilibrium with F-actin, the filamentous form, and the position of this equilibrium is related to differentiation. G-actin serves as an excellent intermediate end-point marker for chemoprevention, and its modulation may serve as a marker for efficacy of retinoid therapy. In carcinogen-exposed worker cohorts, G-actin is a strong marker for exposure. In a pilot chemoprevention study, modulation of G-actin was observed and correlated closely with recurrence. The study of early and late markers in the sequence of carcinogenesis provides a potential set of markers for detecting cancer and developing cancer prevention strategies.

ACKNOWLEDGMENTS

This work was supported, in part, by grants from the National Institutes of Health: 5-U01-CA56981, 5-R01-OH02647, R01-DK45368; and from the U.S. Department of Veterans Affairs: Merit Review 111-34-0132. The authors thank Jean Coffman for her excellent assistance with image analysis, the remainder of the lab group for their hard work, and Martha Hemstreet for her excellent editorial assistance.

REFERENCES

1. Harris CC (1991): Chemical and physical carcinogenesis: advances and perspectives for the 1990s. Cancer Res 51:5023s–5044s.
2. Rao JY, Hemstreet GP, Hurst RE, Bonner RB, Jones PL, Min KW, Fradet Y (1993): Alterations in phenotypic biochemical markers in bladder epithelium during tumorigenesis. Proc Natl Acad Sci U S A 90:8287–8291.
3. Hemstreet GP, Rao JY, Hurst RE, Bonner RB, Jones PL, Vaidya AM, Fradet Y, Moon RC, Kelloff GJ (1992): Intermediate endpoint biomarkers for chemoprevention. J Cell Biochem Suppl 161:93–110.
4. Hemstreet GP, Schulte PA, Ringen K, Stringer W, Altekruse EB (1988): DNA hyperploidy as a marker for biological response to bladder to carcinogen exposure. Int J Cancer 42:817–820.
5. Tanke H, Brussee J, van Driel-Kulker A, van der Burg M, Schelvis-Knepfle C, Jonas U, Ploem J (1983): Results of the Automated Analysis of 328 Bladder Specimens Using the Leyden Television Analysis System (LEYTAS). World J Urol 1:77–81.
6. West SS, Hemstreet GP, Hurst RE, Bass RA, Doggett RS, Schulte PA (1987): Detection of DNA aneuploidy by quantitative fluorescence image analysis: Potential in screening for occupational bladder cancer. In Dillon K, Ho M (eds): "Biological Monitoring of Exposure to Chemicals." New York City: Wiley, pp 327–341.
7. Golden JF, West SS, Shingleton HM, Murad TM, Echols CK (1979): A screening system for cervical cancer cytology. J Histochem Cytochem 27:522–528.
8. McGowan P, Hurst RE, Bass RE, Hemstreet GP, Postier R (1988): Equilibrium binding of Hoechst 33258 and Hoechst 33342 fluorochromes with rat colorectal cells. J Histochem Cytochem 36:757–762.
9. Bass RA, Hemstreet GP, Honker NA, Hurst RE, Doggett RS (1987): DNA cytometry and cytology by quantitative fluorescence image analysis in symptomatic bladder cancer patients. Int J Cancer 40(5):698–705.
10. Hemstreet GP, West SS, Weems W, Echols CK, McFarland S, Lewin J, Lindseth G (1983): Quantitative fluorescence measurements of AO-stained normal and malignant bladder cells. Int J Cancer 31:577–585.
11. Rao JY, Hurst RE, Bales WD, Jones PL, Bass RA, Archer LT, Hemstreet GP (1990): Cellular F-actin levels as a marker for cellular transformation: Relationship to cell division and differentiation. Cancer Res 50:2215–2220.
12. Bi W, Rao J, Hemstreet GP, Fang P, Asal NR, Zang M, Min KW, Ma Z, Lee E, Li G, Hurst RE, Bonner RB, Weng Y, Fradet Y, Yin S (1993): Field molecular epidemiology. Feasibility of monitoring for the malignant bladder cell phenotype in a benzidine-exposed occupational cohort. J Occup Med 35(1):20–27.
13. Rhodes S, Hurst RE, Rollins SA, Jones PL, Hemstreet GP, Detrisac CJ, Thomas CF, Moon RC, Kelloff GJ (1991): DNA ploidy and p21 protein levels in tissue

sections as end-point markers in animal carcinogenesis trials. Biol Monitor 1:61–73.
14. Jones PL, O'Hare C, Bass RA, Rao JY, Hemstreet GP, Hurst RE (1990): Quantitative immunofluorescence, anti-ras p21 antibody specificity and cellular oncoprotein levels. Biochem Biophys Res Commun 167:464–470.
15. Bonner RB, Hemstreet GP, Fradet Y, Rao JY, Min KW, Hurst RE (1993): Bladder cancer risk assessment with quantitative fluorescence image analysis of tumor markers in exfoliated bladder cells. Cancer 72:2461–2469.
16. Hurst RE, Cheung JY, Ashenayi K, Bonner RB, Hemstreet GP (1991): A hybrid image analysis-neural network system for detection of bladder cancer. In: "Proceedings of the Fifth Oklahoma Symposium on Artificial Intelligence," pp 109–112.
17. Rao JY, Hemstreet GP, Hurst RE, Bonner RB, Min KW, Jones PL (1991): Cellular F-actin levels as a marker for cellular transformation: Correlation with bladder cancer risk. Cancer Res 51:2762–2767.
18. Pienta K, Partin A, Coffey DS (1989): Cancer as a disease of DNA organization and dynamic cell structure. Cancer Res 49:2525–2532.
19. Trosko JE, Chang CC, Madhukar BV, Oh SY (1990): Modulators of gap junction function: The scientific basis of epigenetic toxicology. In Vitro Toxicol 3:9–26.
20. Schlussel RN, Droller MJ, Liu BC (1991): Type IV procollagen mRNA regulation: evidence for extracellular matrix/cytoskeleton/nuclear matrix interactions in human urothelium. J Urol 146:428–432.
21. Pienta KJ, Coffey DS (1992): Type IV procollagen mRNA regulation: Evidence for extracellular matrix/cytoskeleton/nuclear matrix interactions in human urothelium. J Cell Biol 49:357–365.
22. Rao KM, Cohen HJ (1991): Actin cytoskeletal network in aging and cancer. Mutat Res 256:139–148.
23. Pollard T, Cooper JA (1986): Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. Annu Rev Biochem 55:987–1035.
24. Wessells NK, Spooner BS, Ash JF, Bradley MO, Luduena MA, Taylor EL, Wrenn JT, Yamada KM (1971): Microfilaments in cellular and development process. Science 171:135.
25. Miron T, Vancompernolle K, Vandekerckhove J, Wilchek M, Geiger B (1991): A 25-kD inhibitor of actin polymerization is a low molecular mass heat shock protein. J Cell Biol 114:255–261.
26. Shyy TT, Asch BB, Asch HL (1989): Concurrent collapse of keratin filaments, aggregation of organelles, and inhibition of protein synthesis during the heat shock response in mammary epithelial cells. J Cell Biol 108:997–1008.
27. Glass JR, DeWitt RG, Cress AE (1985): Rapid loss of stress fibers in Chinese hamster ovary cells after hyperthermia. Cancer Res 45:258–262.
28. Koyasu S, Kadowaki T, Nishida E, Tobe K, Abe E, Kasuga M, Sakai H, Yahara I (1988): Alteration in growth, cell morphology, and cytoskeletal structures of KB cells induced by epidermal growth factor and transforming growth factor-beta. Exp Cell Res 176:107–116.
29. Dadabay CY, Patton E, Cooper JA, Pike LJ (1991): Lack of correlation between changes in polyphosphoinositide levels and actin/gelsolin complexes in A431 cells treated with epidermal growth factor. J Cell Biol 112:1151–1156.
30. Antecol MH (1988): Oncogenic potential in fibroblasts from individuals genetically predisposed to cancer. Mutat Res 199:293–311.
31. Lombardi L, Ballinari D, Bongarzone I (1990): Ultrastructural cytoskeleton alterations and modification of actin expression in the NIH/3T3 cell line after transformation with Ha-ras-activated oncogene. Cell Motil Cytoskeleton 15:220–229.
32. Paterson HF, Self AJ, Garrett MD, Just I, Aktories K, Hall A (1990): Microinjection of recombinant p21rho induces rapid changes in cell morphology. J Cell Biol 111:1001–1007.
33. Felice GR, Eason P, Nermut MV, Kellie S (1990): pp60v-src association with the cytoskeleton induces actin reorganization without affecting polymerization status. Eur J Cell Biol 52:47–59.
34. Marchisio PC, Cirillo D, Teti A, Zambonin-Zallone A, Tarone G (1987): Rous sarcoma virus-transformed fibroblasts and cells of monocytic origin display a peculiar dot-like organization of cytoskeletal proteins involved in microfilament-membrane interactions. Exp Cell Res 169:202–214.
35. Miller CA, Cohen MD, Costa M (1991): Complexing of actin and other nuclear proteins to DNA by cis-diamminedichloroplatinum(II) and chromium compounds. Carcinogenesis 12:269–276.
36. Scheer U, Hinssen H, Franke WW, Jockusch BM (1984): Microinjection of actin-binding proteins and actin antibodies demonstrates involvement of nuclear actin in transcription of lampbrush chromosomes. Cell 39:111–122.
37. Ankenbauer T, Kleinschmidt JA, Walsh MJ, Weiner OH, Franke WW (1989): Identification of a widespread nuclear actin binding protein. Nature 342:822–825.
38. Lamb NJ, Fernandez A, Watrin A, Labbe JC, Cavadore JC (1990): Microinjection of p34cdc2 kinase induces marked changes in cell shape, cytoskeletal organization, and chromatin structure in mammalian fibroblasts. Cell 60:151–165.
39. Lachapelle M, Aldrich HC (1988): Phalloidin-gold complexes: A new tool for ultrastructural localization of F-actin. J Histochem Cytochem 36:1197–1202.
40. Kolber MA, Broschat KO, Landa-Gonzalez B (1990): Cytochalasin B induces cellular DNA fragmentation. FASEB J 4:3021–3027.
41. Loretz L, Reznikoff CA (1988): Clonal growth of normal human uroepitheleal cells. In Vitro Cell Dev Biol 24: 333–342.
42. Bookland E, Swaminathan S, Oyasu R, Gilchrist K, Lindstrom M, Reznikoff CA (1992): Tumorigenic transformation and neoplastic progression of human uroepithelia cells after exposure in vitro to 4-aminobiphenyl or its metabolites. Cancer Res 52(6):1606–1614.
43. Pratt CI, Kao CH, Wu SQ, Gilchrist KW, Oyasu R, Reznikoff CA (1992): Neoplastic progression by EJ/ras at different steps of transformation in vitro of human uroepithelial cells. Cancer Res 52:688–695.
44. Bremer JW, Busch H, Yeoman LC (1981): Evidence for a species of nuclear actin distinct from cytoplasmic and muscles actins. Biochemistry 20:2013–2017.
45. Liebert M, Wedemeyer GA, Stein JA, Washington RW, Jr., Flint A, Ren LQ, Grossman HB (1989): Identification by monoclonal antibodies of an antigen shed by human bladder cancer cell. Cancer Res 49:6720–6726.
46. Sidransky D, Frost P, von Eischenbach AC, Dyasu R, Preisinger AC, Vogelstein B (1992): Clonal origin bladder cancer. N Engl J Med 326(11):759–761.