Review

Saturation Density of Skin Fibroblasts as a Quantitative Screen for Human Cancer Susceptibility

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Abstract

Genomic analysis of human cancers reveals a large number of genetic changes per cell that presumably underlie development of the disease. The complexity of these changes that differ from one type of cancer to the other and from patient to patient with the same type of cancer raises questions about the feasibility of genomic analysis as an indicator of susceptibility to cancer. However, skin fibroblasts (SF) from individuals in families with heritable forms of cancer, and from cancer-bearing individuals, show correlation with a significant increase in saturation density (SD), as well as other neoplasia-related properties. Procedures are described for amplifying and quantifying differences in SD on the basis of studies of spontaneous transformation in the NIH 3T3 line of mouse fibroblasts. It is proposed that such procedures be evaluated as quantitative screens for susceptibility to cancer in the general population. (Cancer Epidemiol Biomarkers Prev 2009;18(9):2366–72)

Introduction

The large number of genetic alterations that underlie the development of cancer in humans (1-3) make it unlikely that cancer susceptibility can be accurately gauged by genomic analysis (4-6). Living cells, however, integrate the phenotypic effects of many genes, and long-standing evidence is presented that increased saturation density (SD, the maximum number of cells in a culture, limited by contact inhibition and the concentration of serum in the medium) of skin fibroblasts (SF) is displayed both in the case of the high penetrance of a single mutated gene in cancer-prone families (7, 8), and of multiple mutated genes in "sporadic" breast (9) and other cancers (10). Increases in SD result from selection of cells under contact inhibition of mutants that mainly arise during the replication of cells (11, 12). Sensitive, quantitative methodologies are described that combine mutation and selection of SF to detect variation in SD, and are proposed as screens in human populations for susceptibility to cancer.

Complexity of Genetic Changes in Human Cancer

It is generally accepted that genetic changes underlie the development of cancer in humans and animals (13), although the required number of such changes is contingent on the system examined. Infection of cells, with one particle of Rous sarcoma virus containing a single transforming gene can result in tumor production *in vivo* (14) or transformed focus production *in vitro* (15), but that capability is due to the presence of a strong viral promoter for expression of that gene (16). Transfection of NIH 3T3 cells with an activated *ras* gene from a human cancer advances their state of transformation (17), but that is because the NIH 3T3 cells were already partially transformed (18). More recently, advances in genomic analysis have permitted sequencing of thousands of genes from naturally occurring human epithelial cancers that underlie the development of cancer (1-3). Such observations occasioned the Editorial comment in *Nature* that "a tumor cell is a genetic disaster area littered with mutations that differ not only from one type of cancer to the next, but from one patient to the next" (5, 6). The altered genes include a small number called "mountains" that are commonly mutated in particular cancers, and a much larger number of "hills" that are mutated at low frequency per tumor (19). It is likely that some of the early common mutations of the "mountains" increase fitness of cells more than the average, allowing a small initiating lesion to grow into an intermediate size lesion (20). Once a growth reaches this size, a large number of mutations in the "hills," with small fitness advantages can accumulate and drive tumor progression to a cancer. It is the "hills" rather than the "mountains" that dominate the tumor landscape (19).

Sequence analysis reveals that a large fraction of the tumor-associated genetic alterations are point mutations (21), illustrating the complexity of human cancer. No single mutated gene lies at the heart of the major cancers, which complicates the prospect for new targeted drug therapies (5) and genetic tests for susceptibility to cancer. The complexity is increased by the number of homozygous deletions and amplifications of the tumors (22). There are at least 12 major deletions or amplifications per cell and an average of 17 per tumor. These do not

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include detection of very small (<20 kb) amplifications or deletions, nor do the methods allow detection of structural changes such as translocations or of epigenetic alterations. It was suggested that the degree of complexity could be reduced because most of the mutated genes involved a comparatively small number of biochemical pathways (21). But that does not consider that "all genes studied with any care have pleiotropic effects," i.e., which influence multiple aspects of the phenotype (23), which would add further complexity to the analysis.

The quantitative problem in cancer resembles the one encountered in the search for genetic variants associated with height differences (24). More than 40 such variants were found, but together they accounted for little more than 5% of height's variability. Yet height, as estimated from the height of parents, is 80% to 90% heritable. A similar situation exists for autism, schizophrenia, obesity, diabetes, and heart disease. There may be moderately penetrant genes that are rare enough that they are missed by genome-wide association studies, and there may be many more frequent variants that have such a low penetrance that they cannot statistically be linked to the problem under study. Thousands of such variants may be needed to get near 80% or 90% heritability. One geneticist remarked that "Taken to the extreme, practically every gene in the genome could have a variant that affects height" (24), which might apply to the other conditions mentioned above.

It has long been known that nongenetic factors have high attributable risks in cancer, often at least 80% or 90%, even when the etiological factors are not clear (25). Modifiable behavioral factors, including specific aspects of diet, overweight, inactivity, and smoking, account for more than 70% of colon cancer. Highly penetrant mutations associated with conspicuous clustering of diseases within families are rare, and seem to account for less than 5% of major cancers. As noted above, the majority of the genetic contributions to cancer risk results from a large number of low penetrance variants in gene sequence (19, 20). Typically, these mutations have relative risks less than two, and therefore are not strong enough to cause obvious clustering of disease within families (25).

Although the population-attributable risk for any one of these polymorphisms may be small, many different polymorphisms could collectively result in a substantial attributable risk because of genetic factors. However, classic heritability studies, such as investigation of twins, do provide some insight into their role in the etiology of cancer. Notably, there was a very large scale study in Scandinavian countries of differences in the incidence of cancers at 26 sites among monozygous and dizygous twins (26). It showed a high proportion of susceptibility (27%-42%) to colorectal, breast, and prostate cancers that was accounted for by genetic effects that were statistically significant (i.e., for which the 95% confidence level did not include zero). Cancers at 14 other sites exhibited higher concordance between monozygotic twins than that between dizygotic twins, indicating a genetic contribution, but at lower confidence levels. No concordant pairs were observed at the remaining nine sites, indicating very little genetic contribution to susceptibility. However, it seems unlikely that individual genetic variants will be useful for screening and risk identification in human cancer because the great majority confer only small relative risks (25) that weakly predict the risk, and combining them into a risk score would be a more effective predictor of cancer. This could be done for genes concerned with estrogen levels, which are moderately good predictors of breast cancer risk and could provide much better discrimination than any single genotype. That information, however, may be less useful than simply measuring serum estrogen levels, which summarize all the genetic and environmental determinants (25). Evaluation of proposed genetic trials indicated that they would be both expensive and protracted, involving 10,000 to 30,000 individuals, and therefore considered impractical (4).

Growth Abnormalities in SF from Individuals of Families Genetically Predisposed to Colorectal Cancer

It is well known that normal fibroblasts undergoing transformation in culture alter their growth behavior in a more or less progressive fashion. These changes include increases in SD (ref. 27) (the maximum number of cells in a culture, limited by contact inhibition and the concentration of serum in the medium), ability to proliferate in low concentrations of serum, colony formation in suspension, and tumor formation in experimental animals (27, 28). These graduated changes raise the question of whether the early stages of progression, namely differences in SD and growth in low concentrations of serum, can be used to identify individuals who are susceptible to the development of cancer. Tests on SF obtained from individuals genetically predisposed to colorectal cancer (ACR/FAP hereditary adenomatosis of the colon and rectum, also known as familial adenomatous polyposis) do indeed exhibit such behavior. This condition arises from a dominant mutation in the *APC* gene (29). SF obtained from symptomatic individuals from families bearing this mutation exhibit growth behavior that distinguishes them from SF of normal individuals in two characteristics, namely an ability to proliferate in low concentrations of fetal bovine serum (FBS) (ref. 7), and a capacity to multiply to significantly higher than normal SD in conventional concentrations of FBS (8). The asymptomatic progeny of symptomatic members of ACR/FAP families would be expected to be, and are, mixed among those that can and cannot proliferate in low FBS (Table 1A), because these progeny consist of carriers and noncarriers of mutations in one allele for the *APC* gene. Two hits are represented by the tumor cells in symptomatic patients. The normal SF from all normal individuals, including spouses of symptomatic ACR/FAP individuals and normal volunteers, fail to multiply in low FBS, as do cell strains from normal individuals. The results, therefore, indicate an association between mutations of the *APC* gene that underlie adenomatosis of the colon and rectum, and the ability of SF from such individuals to proliferate in low serum.

A correlation was made between the ability of SF to multiply in low FBS and SD in high FBS. All the SF samples that could proliferate in low FBS multiplied to higher saturation densities in high FBS than did the samples from the low FBS negatives (Table 1B). An even larger difference between these groups was found when the rate of ^{3}H-thymidine incorporation into DNA was measured

Table 1.

A. Serum requirements of SF growth from ACR/FAP families and controls

Group	No. of subjects	1% FBS* ± SEM	*P*	15% FBS* ± SEM	*P*
I. Symptomatic ACR/FAP	13	2.68 ± 0.28	<0.001	6.57 ± 1.53	N.S.
II. Asymptomatic ACR/FAP progeny					
Low serum positive	10	2.28 ± 0.13	<0.001	4.78 ± 0.84	N.S.
Low serum negative	4	0.95 ± 0.05	N.S.	4.64 ± 1.56	N.S.
III. Normals					
Spouses of symptomatic ACR/FAP	5	0.96 ± 0.03	-	6.30 ± 1.54	-
Healthy volunteers	5	0.96 ± 0.02	-	3.73 ± 0.87	-
IV. Normal cell lines	13	0.95 ± 0.02	-	2.98 ± 0.13	-

NOTE: *P* values represent group analyses and express the probability of obtaining a difference with respect to the average values of groups III and IV. Adapted from Pfeffer et al. (7).
Abbreviations: N.S., not significant; SEM, standard error of the mean.
*Fold change in cell numbers between days 1 and 5.

B. SD of SF from ACR/FAP families and controls

Group	No. of Subjects	Cell Number × 10^{-4}/cm^2 †
I. Symptomatic ACR/FAP	12	8.91 ± 0.94
II. Asymptomatic ACR/FAP progeny		
Low serum positive	7	7.72 ± 1.13
Low serum negative	3	3.68 ± 0.28
III. Normals		
Spouses of symptomatic ACR/FAP and normal cell lines	12	3.59 ± 0.47

Adapted from Kopelovich et al. (8).
†SD in 15% FBS.

after SD was reached (data are not shown here). That finding suggests the two characteristics, i.e., ability to proliferate in low FBS and to achieve significantly higher saturation densities, reflect the same properties of cells bearing hereditary mutations in one or both alleles of the *APC* gene.

Individuals with Neoplasms Not Related to Known Familial Cancer History

The relation of SD to the occurrence of breast cancer that had no known familial association is shown in Table 2A. SF were taken from patients who had breast cancer and those who had benign breast lesions (9). They were passaged in cell culture according to a 1:2 split regime, and some were shifted after a few passages to a parallel series of overcrowded 1:1 passages. The saturation densities of the serial passages under the two conditions were compared with each other (Table 2A). All the SF samples taken from the breast cancer patients passaged in a 1:1 passage regime reached 40% or higher SD than those passaged in a 1:2 passage regime. All but one of the SF taken from patients with benign breast lesions reached a low SD under both passage conditions. The exception in which there was a large increase in SD of the SF under overcrowded conditions was from a patient whose mother was affected with breast cancer. That patient developed a malignant lesion 3 years later. The results could be taken to indicate that breast cancer is linked to a genetic background favoring the development of the neoplasia. This interpretation is reinforced by the very high incidence of breast cancer in both members of pairs of monozygotic twins (30).

Using a labeling technique related to SD, studies were done with SF from patients with breast and other cancers as well as normal controls (10). SF in each case were grown to their SD and labeled with ^{3}H-thymidine on the second day of the plateau phase. The incorporation of the label into SF from patients with breast cancer, melanoma, hepatocarcinoma, and osteosarcoma were significantly higher than any of those from normal controls (Table 2B). In two instances the detection of the high-labeling capacity preceded the discovery of the disease. It was concluded that the cancer patients constituted a group more sensitive to environmental factors and/or to radiotherapy or chemotherapy and therefore more prone to develop primary or secondary neoplasia. The authors suggested that at least in some instances cancer is a systemic disease that permits the assays in SF employed in Table 2 to be used as a screening for patients with high risk of cancer. The altered fibroblasts might also provide a microenvironment favorable to cancer development (31, 32).

Other Abnormalities of SF Correlated with Human Cancer

A variety of abnormalities other than increased SD or growth in low serum have been described in SF from individuals either bearing cancers or with familial conditions associated with cancer (Table 3). They include increased susceptibility to transformation by infection with SV40 virus, or spontaneously occuring chromosome abnormalities, growth in suspension, and abnormal migration into collagen. It was noted that prolonged maintenance at confluence of fibroblasts derived directly from a lung carcinoma promoted the development of foci of cells with altered morphology (33). Overcrowding increased the SD of SF

[1] T. Ignatova, personal communication.

Table 2.

A. Increase in SD in overcrowded cultures after first 1:1 split

SF from Patients	
With malignant breast lesions	With benign breast lesions
% change*	% change*
+ 40	+ 10
+ 50	+ 8
+ 50	+ 58†
+ 45	−10
+ 50	+ 10
+ 40	−3
+ 70	−3

NOTE: Adapted from Azzarone et al. (9).
*After several passages of SF at a 1:2 split, they were passaged in parallel at 1:1 and 1:2 splits, and the SD change of the 1:1 split determined.
†Mother of this patient was affected with breast cancer. The patient developed breast cancer 3 y later.

B. Percent of postconfluent SF labeled with ^{3}H-thymidine

	SF from Cancer Patients	SF from Noncancer Patients	
	Percent Labeled	Percent Labeled	
Breast cancer	22 ± 3.3‡	7.0 ± 1.3‡	3.0 ± 1.2
Breast cancer	25 ± 3.7	7.0 ± 1.1	4.0 ± 0.9
Breast cancer	28 ± 3.7	3.0 ± 0.9	3.3 ± 1.3
Melanoma	30 ± 4.5	3.0 ± 0.6	7.0 ± 1.3
Hepatocarcinoma	30 ± 4.5	1.6 ± 1.0	
Osteosarcoma	28 ± 3.0	1.5 ± 0.7	

Adapted from Azzarone et al. (10).
‡Cells were labeled with ^{3}H-thymidine on second day of the plateau phase of growth curves and the percent labeled nuclei determined.

aberrations were produced in SF from Li-Fraumeni syndrome after long term contact inhibition at confluence.[1] Of special interest is the tumorigenic transformation of SF from ACR/FAP individuals treated with tumor promoter alone, which implies that the SF were initiated cells (Table 3). These observations indicate that selection of cells at confluence favors the expression of diverse neoplasia-related properties of SF from cancer-prone individuals. Altered proliferation and differentiation properties have recently been reported in primary mammary epithelial cells from BRCA1 mutation carriers, who have a high probability of developing breast cancer (35).

Amplification of Increases in SD

No sustained systematic attempt was described in the above experiments to optimize the techniques for demonstrating increased SD of SF from individuals susceptible to or bearing cancer. The parameters for such a methodology, however, can be inferred from studies of spontaneous 3T3 line came into wide prominence as the cell of choice for demonstrating the transforming capacity of a mutated gene from a human cancer (36). The probable reason for the unique sensitivity of this cell line to transformation by a single gene was that it was already in an incomplete state of transformation, as indicated by the appearance of small numbers of transformed foci in confluent cultures of untreated cells in the presence of high concentrations of bovine calf serum (CS) (refs. 37, 38). Reducing the CS concentration to low levels prevented the formation of the transformed foci, but allowed their eventual development in size and increasing number in multiple rounds of confluence, preceded by progressive increases in SD (39).

Variations of serum concentrations and time at confluence pointed to regimens for accelerating and maximizing transformation. The results of varying the conditions for obtaining progressive increases in SD are presented in Table 4. The saturation densities in the first round of confluence (1° assay) were directly proportional to the concentration of serum used in that assay and are slightly

Table 3. Other abnormalities of SF correlated with human cancer conditions

Year	Cancer-Related Condition	SF Abnormality in Culture	Reference
1966	Fanconi's anemia	>10-fold increase in transformed foci from SV40 virus infection	(47)
1976	Lung cancer	Abnormal ploidy, growth in suspension, foci in monolayers, spontaneous transformation	(48)
1979	ACR/FAP	Tumorigenic transformation induced by tumor promoter alone	(49)
1980	ACR/FAP	Growth in suspension after mutagen treatment	(50)
1983	ACR/FAP	Chromosome abnormalities in early cultures	(51)
1984	Breast cancer	Increased hyperploidy from patients with, or at risk for, breast cancer	(52)
1985	Breast cancer, melanoma, ACR/FAP, retinoblastoma, Wilm's tumor	Migrate into collagen like fetal fibroblasts rather than adult fibroblasts	(53)
1989	Li-Fraumeni Syndrome	Morphological transformation, chromosome abnormalities	(54)

Table 4. Effect of variation in calf serum concentration and time on SD of NIH 3T3 cells in 1° assay, and its relation to SD in subsequent serial passages at constant low serum and time

1° Assay in	SD × 10^{-5} Cells per Culture		
	Assay	2 Week 1° Assay	3 Week 1° Assay
2% CS	1°	*4.28 ± 0.21*	*4.68 ± 0.06*
	2°	4.60 ± 0.21	5.32 ± 0.12
	3°	7.25 ± 0.29	8.35 ± 0.32
	4°	8.73 ± 1.32	12.97 ± 0.31
5% CS	1°	*9.57 ± 0.48*	*11.30 ± 0.33*
	2°	5.75 ± 0.31	7.27 ± 0.35
	3°	7.95 ± 0.37	10.30 ± 0.71
	4°	9.37 ± 0.27	17.15 ± 1.2
10% CS	1°	*21.35 ± 0.46*	*21.90 ± 1.27*
	2°	7.0 ± 0.27	15.3 ± 9.4
	3°	11.24 ± 5.4	64.8 ± 8.5
	4°	40 ± 17.9	N.D.

NOTE: NIH 3T3 cells were seeded in quadruplicate lineages in a 1° assay for each of the indicated variations in CS concentration and time, and then passaged serially in constant low CS for 2 wk in the 2°, 3°, and 4° assays. Saturation densities were determined at each passage, with the 1° assay results shown in italics.
Abbreviation: N.D., not done.
Adapted from Rubin et al. (11, 39).

increased by an additional week of incubation. All succeeding rounds of confluence (2°, 3°, and 4° assays) were incubated in the lowest concentration of serum for a 2-week period. The higher the initial concentration of serum in the 1° assay, the larger the total number of cell divisions with attendant mutations to be selected for overgrowth at confluence. Increasing the concentration of serum in the first round accelerated the increase of SD in subsequent rounds of confluence under constant conditions, thereby increasing the speed and sensitivity of the assay for transformation (11, 39). The dilution of cells to subconfluent densities at each passage increased proliferation and thereby mutation, which enriched the selection process at confluence. The longer the cells were incubated in the first cycle, the further they increased in SD during the following cycles (Table 4). The largest increase by far of SD (and of transformed foci) in the later assays (39) was induced by extending the time of the 1° assay in the highest concentration of serum. This result suggests that selective growth is most efficient within local groups of transformed cells when contact inhibition by nontransformed cells is minimized. Eventually, discrete transformed foci appear from a minority of the cell population after an accumulation of progressive mutations. This observation showed that SD is an early sign of transformation, and indicated that transformed focus formation is a continuation of the mutation-selection process that drove up SD. But it was particularly clear when the entire process was extended in time by using the lowest concentration of serum in the 1° assay (as well as in the 2°, 3°, and 4° assays) that reliable increases in SD of less than 10% can be detected long before even the smallest discrete foci of overgrowth appear (11, 39). It is noteworthy that a subline of uncloned NIH 3T3 cells that were relatively resistant to spontaneous transformation exhibited little change in SD upon multiple serial rounds of confluence (40).

Proposals for Amplifying and Quantitating Differences in SD of SF

SD has been the most precise and quantitative indicator of difference reported between SF from cancer-prone and normal individuals (Tables 1 and 2). The results however are given essentially as positive or negative, with little evidence of degrees of difference in SD. If SD of SF were to be applied as a screening method for differences in degree of susceptibility to cancer, the range of differences in SD of the SF would have to be expanded, their intermediate values accurately determined, and their relation to cancer susceptibility evaluated. Methods have in fact been developed in studies of spontaneous transformation of the mouse NIH 3T3 line of fibroblasts for amplifying saturation densities, obtaining their intermediate values, and evaluating the reproducibility of the measurements (39). Those methods consist of serial rounds of a combination in each round of (a) proliferation of the cells at subconfluent densities to encourage mutation, and (b) prolonged incubation at high densities to allow selection of variants that proliferate under contact inhibition (Table 4).

Optimal methods for achieving the desired goals are based on the following considerations. Proliferation, and therefore mutation, is maximized in high concentrations of serum both at the subconfluent densities, and after selection begins at the higher density. Because SD is itself proportional to serum concentration, the increased total number of cells in high serum implies a proportional increase in the number of mutations. However, variation in the intrinsic capacity of SF from different individuals for increasing SD under selection might be partially compromised in high serum because those populations with low capacity would nevertheless be driven to relatively high saturation densities in high serum (Table 1A). Therefore detection of differences in SD at each subculture would optimally be determined in lower concentrations of serum, e.g., 2% serum in Table 4. The optimal high and low concentrations of serum for discriminating variation in SD of SF among human donors would have to be determined, as they may differ from those established for the NIH 3T3 cell line. A suggested schematic for screening SF from individuals for SD is shown in Fig. 1. It differs from that in Table 4 by running serial rounds of prolonged confluence in high CS that would give some measure of SD and provide the maximum number of mutants for increased SD. At each passage level, a set of the cells would be passaged once at a lower serum concentration to provide finer discrimination among SDs.

There is an alternative method for accelerating increases in SD. Low densities of the NIH 3T3 cells can be passaged every 2 to 3 days in a low concentration of serum that allows their continuous exponential proliferation at a slightly reduced rate, but imposes a sharper reduction in SD if time were extended to form postconfluent monolayers (11). When a fraction of the cell population is set aside in each of the successive short term passages, and permitted to grow to SD in the low serum concentration, there is a rapid, progressive increase in SD (Fig. 5 in ref. 11). A potential problem in applying this method to primary cultures of SF is that primary cultures tend to grow poorly at low densities even in high serum concentrations. That problem may be overcome by using medium that has been conditioned by several days of incubation with higher densities of cells (41).

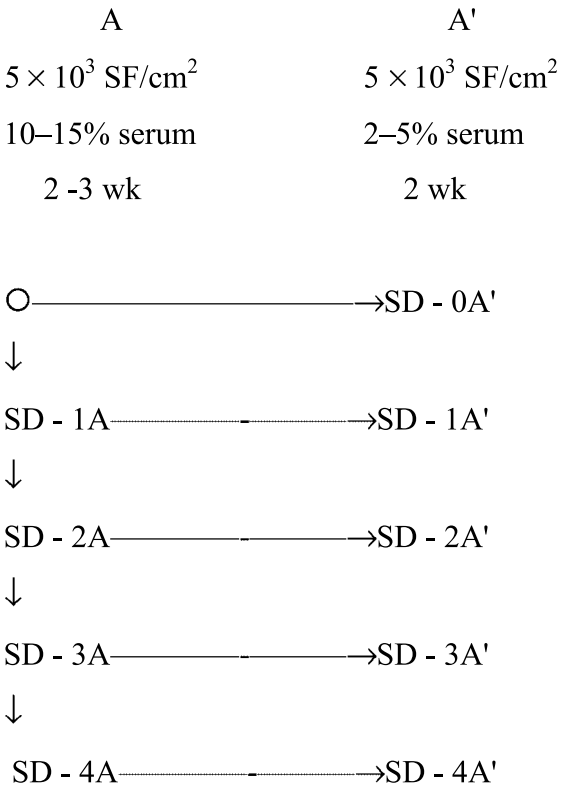

Figure 1. Procedure for optimizing SD of SF as a quantitative screen for cancer susceptibility. Cultures of SF are established from subepidermal biopsies of human skin by conventional methods (7, 9). The SF are divided in two (or four) parallel lineages, 5×10^3 cells/cm^2, in 10% or 15% serum for 2 or 3 wk; and in 2% or 5% serum for 2 wk with frequent medium changes. The cells from the high serum series are trypsinized and counted (SD - 1A) and reseeded in exactly the same manner for serial SD determinations (SD - 2A, - 3A, - 4A). The SF seeded in 2% or 5% are also counted for SD - 0A'. Each of the serial SD determinations in high serum is also seeded in the low serum for a more discriminating SD - 1A', - 2A', - 3A', -4A'. The slopes of both series are determined. All SD values are used to rank the SF from each donor. The initial studies can be done with (a) inbred mouse strains of known risk for cancer; (b) humans from families of known cancer risk; and (c) patients with cancer. If thus validated, the method would be applied as a screen for cancer susceptibility in the general population.

Although the serial assays of SD would require 2 to 3 months, they are not labor intensive, because they involve only medium changes with subculturing and counting cells every few weeks. A single technician can manage many specimens in parallel. The methodology can be carried out in any cell culture facility, and the cost is low, probably no more than a few hundred dollars per sample as compared with $100,000 per sample for the alternative of full genomic analysis.[2] In addition, much can be learned about the biology of the earliest stages of neoplastic development, including, eventually, those genes that contribute to its progression when lowered costs would make genomic analysis practicable.

[2] B. Vogelstein, personal communication.

Conclusions

Increased SD is associated with loss of contact inhibition and decreased capacity to suppress the expansion of initiated or transformed cells into clonal tumors (12). It seems to be the cell culture equivalent of the hyperplasia *in vivo*, which is a common precursor to neoplasia (42-44). Increased SD is a highly accurate and simply quantified assay. It is the earliest neoplastic change we can reproducibly detect, even at very low levels, in progressive transformation (see Table 4, started from 1° assays in 2% CS). SD increase, therefore, constitutes the first measurable stage of initiation. The fact that SF in cancer prone families and in many cases of sporadic breast and other cancers have properties of initiated cells suggests that the field of epithelial cells surrounding a fully initiated homotypic epithelial cell has a reduced capacity to suppress its clonal expansion. Indeed it has been shown that epidermal cells that exhibit early *in vitro* characteristics of initiated cells, but form normal skin in grafts, have lost their capacity to suppress clonal expansion of fully initiated papilloma cells (45). This observation would also be consistent with twin studies that indicate "a very high proportion and perhaps a majority of breast cancers arise in a susceptible minority of women" (30). The abnormal growth behavior of SF in cancer-prone individuals has suggested that at least in some cases cancer can be considered a systemic disease (10, 46), and that this difference in the behavior of SF cells from such individuals may be a practical basis for prevention, diagnosis, and management of the disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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