receiving vehicle (PBS), 5α-dihydrocortisol, 5β-dihydrocortisol, or 5β-tetrahydrocortisol alone had high concentrations of glucocorticoid receptor remaining in the cytosol (approximately 400 fmole per milligram of cytosolic protein), indicating a lack of glucocorticoid activity. In animals receiving active glucocorticoids (1 percent cortisol or 0.1 percent dexamethasone-21-phosphate), the glucocorticoid receptor concentration in the cytosol was reduced to about 100 fmole per milligram of cytosolic protein. These values are similar to those found earlier (*4*). Experiments with dilutions of cortisol and dexamethasone-21-phosphate showed their threshold values for nuclear translocation (the lowest dose producing a measurable effect) to be 0.02 and 0.003 percent, respectively. The addition of 5β-dihydrocortisol (0.1 or 1.0 percent) to these threshold concentrations of cortisol and dexamethasone potentiated their glucocorticoid activity (Fig. 2). 5α-Dihydrocortisol and 5β-tetrahydrocortisol were inactive as potentiators, indicating the specificity of the 5β-dihydrocortisol effect. Dihydrocortisol has weak mineralocorticoid activity and may potentiate low levels of aldosterone (*6*).

Our earlier finding that 5β-dihydrocortisol accumulates in cells cultured from trabecular meshwork specimens (outflow region) from patients with POAG and our present observation that this metabolite potentiates glucocorticoid activity suggest that the ocular hypertension of these patients and their sensitivity to exogenous glucocorticoids may be related to an altered local metabolism of cortisol that leads to the accumulation of this metabolite.

BERNARD I. WEINSTEIN
GARY G. GORDON
A. LOUIS SOUTHREN
New York Medical College, Valhalla 10595-1691

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Normal Cells of Patients with High Cancer Risk Syndromes Lack Transforming Activity in the NIH/3T3 Transfection Assay

Abstract. *Oncogenes capable of transforming NIH/3T3 cells are often present in human tumors and tumor cell lines. Such oncogenes were not detected in normal fibroblast lines derived from patients with several clinical syndromes associated with greatly increased cancer risk. Thus, germ-line transmission of these oncogenes does not appear to be the predisposing factor responsible for these high cancer risk syndromes.*

The development of DNA-mediated transfection techniques (*1*) and cell lines capable of detecting transforming DNA sequences (*2*) has led to the detection of oncogenes in many human tumors and tumor cell lines (*3*, *4*). These genes have arisen from well-conserved normal cellular genes or proto-oncogenes. Genetic changes as small as point mutations (*5*), as well as DNA rearrangements such as transpositions (*6*), gene amplification (*7*), and chromosomal translocations (*8*), have all been implicated in oncogene activation. Three related oncogenes have been detected at high frequency in the NIH/3T3 transfection assay (*4*, *9*, *10*). Each of these genes is related to the *ras* family of *onc* genes (*11*), cellular sequences that have been transduced by retroviruses to form Harvey, BALB, and Kirsten murine sarcoma viruses. The *onc* genes of these viruses are essential for their tumor-inducing potential.

While most forms of cancer occur sporadically, clinical observations have revealed the existence of certain familial aggregations of cancer involving specific target organs. Other familial syndromes are associated with a strikingly increased risk of cancer characterized by onset at an early age and tumors affecting tissues of more than one developmental type (*12*). Such clinical syndromes provide the opportunity to test whether the germline transmission of oncogenes capable of registering in the NIH/3T3 transfection assay might account for these patients' genetically determined high risk of cancer.

High molecular weight DNA was prepared from fibroblast cultures derived from skin of patients with various clinical conditions associated with a high risk of cancer (Table 1). The largest group comprised affected individuals from five families with Gardner's syndrome. This syndrome is associated with a dominant trait for susceptibility to colon cancer as well as a striking familial predisposition to benign neoplasms of bone, soft tissue, and skin (*13*). Essentially 100 percent of such affected individuals develop colon cancer in the absence of clinical intervention (*13*). In addition, we studied fibroblasts from tumor-bearing members of families with site-specific tumor aggregations with apparent autosomal transmission (*12*, *14*). Patients affected with other syndromes associated with strikingly increased cancer risk, including xeroderma pigmentosum and von Recklinghausen's syndrome, were studied as well (*12*).

DNA transfection was performed as described earlier (*10*, *15*). We observed transforming activity with 20 to 33 percent of colon or lung carcinoma DNA samples analyzed (Table 1). These results compare favorably with the reported prevalence of transforming genes in cell DNA's from human tumors as detected with the NIH/3T3 assay (*3*, *4*, *10*). The transforming activity associated with positive tumor cells ranged from 0.2 to 1.3 focus-forming units per plate. Representative transformants were also tested and shown to contain human repetitive sequences (*16*), confirming that each had taken up exogenous human DNA. In contrast, we detected no evidence of transforming DNA sequences in any of the skin fibroblast lines from patients with the high cancer susceptibility syndromes analyzed. To ensure that each of the fibroblasts was adequately tested, more than 1000 μg of high molecular weight DNA was tested in each case, with analysis conducted in at least two separate experiments.

Antiserums that recognize the 21,000-dalton (p21) gene products of both mouse and human homologs of the *ras* genes are available (*17*). Moreover, in human tumor cells that contain activated *ras* oncogenes, the mobility of the p21 protein can be altered (*5*). Therefore, we also considered whether any abnormality could be detected in the physical characteristics or level of expression of these proteins in any of the human fibroblast lines analyzed. By use of monoclonal antibodies that detect human p21, we were able to demonstrate a new p21 species with altered electrophoretic mobility in the T24 bladder carcinoma cell line (*5*). We also examined seven additional human tumor cell lines known to contain activated *ras* oncogenes. Three of these showed either an additional p21 species or an increase in the relative

intensity of one p21 species present in the cells. In contrast, none of 18 fibroblast lines representing each of the cancer susceptibility syndromes in our studies revealed any protein species distinguishable in electrophoretic mobility or quantity from those observed in normal human fibroblasts.

The detection of a single-copy transforming gene by the NIH/3T3 transfection assay requires its presence in a large fraction of the cell population (*3–5*, *9*, *10*). For heterogenous fibroblasts, this would require oncogene activation within the germ line or very early in embryonic development. It is conceivable that an activated oncogene might be present but suppressed by mechanisms such as DNA methylation in fibroblasts of high-risk patients. This argument would necessitate a highly specific suppression of the activated allele, since p21 was readily detected in all of the fibroblast lines analyzed. Our failure to detect oncogenes of the type commonly activated in human cancer cells in fibroblasts of patients with various high cancer risk syndromes argues strongly against the germ-line transmission of such oncogenes in these individuals. It would seem even more improbable that patients with the sporadic occurrence of single tumors would show the germ-line transmission of such oncogenes. Although there has been a report of an activated cellular oncogene in normal cells from such a patient (*18*), these data were subsequently found to be due to an artifact (*19*).

We have also analyzed tumor tissues from two individuals with Gardner's syndrome and from one patient with familial breast cancer. None of these contained oncogenes capable of registering in the NIH/3T3 transfection assay. These findings further support the concept that the germ-line transmission of oncogenes detectable in the NIH/3T3 transfection assay does not account for these patients' high susceptibility to develop cancer. Nonetheless, patients with familial cancer syndromes inherit factors that predispose to malignancy. Examples of such heritable factors may include oncogenes not detectable by transfection or deficiencies in DNA repair, carcinogen metabolism, or immunologic surveillance.

Table 1. Prevalence of transforming genes detected in sporadically occurring tumors and in skin fibroblasts from patients with high cancer risk syndromes. Human fibroblast cultures were initiated from sterile normal skin biopsies by standard procedures or provided by other investigators. The lung and colon carcinoma cell lines used were derived in our laboratory or were obtained from the American Type Culture Collection. Cells were grown in Dulbecco's modified essential medium supplemented with 15 percent fetal calf serum and antibiotics. Cells (3×10^7 to 5×10^7) were lysed in 0.5 percent sodium dodecyl sulfate containing 10 m*M* tris, 2 m*M* EDTA, and 150 m*M* NaCl and treated overnight with proteinase K (0.2 mg/ml). Proteins were extracted with a mixture of phenol, chloroform, and isoamyl alcohol (25:24:1) and then with chloroform and isoamyl alcohol (24:1). DNA was precipitated with ethanol, dried, and suspended in 1 m*M* tris and 0.2 m*M* EDTA. The DNA transfection assay was performed as described (*10*, *15*). Briefly, NIH/3T3 cells were plated in 10-cm petri dishes (Costar) at 1.3×10^5 cells per plate for 20 hours before transfection. DNA (80 μg) was suspended in 0.25*M* $CaCl_2$ and precipitated by addition to an equal volume of 50 m*M* Hepes, 280 m*M* NaCl, and 1.5 m*M* Na_2PO_4 during gentle nitrogen bubbling. After 30 to 60 minutes, precipitates were resuspended and added to the NIH/3T3 cell monolayer. Precipitates were removed 20 hours later. Culture media were changed twice weekly, and focus formation was scored at 14 to 21 days. Genomic DNA from the human bladder cancer cell line T24 was used as a positive control. T24 DNA yielded 5 to 15 focus-forming units per plate. Other transfection-positive human lung and colon carcinomas gave 0.2 to 1.3 focus-forming units per plate. The positive DNA's of individual fibroblast cell lines were tested in at least two separate experiments, with a total of 16 plates analyzed. Fibroblast lines not specifically cited were developed in our laboratory.

Cell line	Positive DNA's (No.)	Patients tested (No.)	Reference for source
Sporadically occurring tumors			
Colon carcinoma	2	10	(*20*)
Lung carcinoma	5	15	(*20*)
Skin fibroblasts in high cancer risk syndromes			
Familial polyposes			
Gardner's syndrome			
A*	0	6 (0)†	(*21*)
B	0	3 (0)	(*22*)
C	0	4 (0)	(*23*)
D	0	1 (0)	(*24*)
E	0	1 (0)	
Adenomatosis of colon and rectum			
A	0	2 (0)	(*25*)
Site-specific familial tumor aggregation			
Skin and connective tissue	0	3 (2)	(*24*)
Mammary and gastric cells	0	1 (1)	(*24*)
Colon	0	1 (1)	(*21*)
Colon and endometrium	0	1 (1)	(*21*)
Osteogenic sarcoma	0	2 (2)	
Miscellaneous syndromes			
Xeroderma pigmentosum			
A	0	1 (0)	(*24*)
B	0	1 (0)	(*24*)
C	0	1 (0)	
von Recklinghausen's syndrome	0	1 (1)	(*24*)
Down's syndrome	0	1 (0)	

*Each letter signifies a different family in which fibroblasts from affected members were analyzed. †The number in parentheses indicates fibroblast lines from tumor-bearing individuals from each of the genetically determined cancer susceptibility syndromes.

SAMUEL W. NEEDLEMAN
YASUHITO YUASA
SHIV SRIVASTAVA
STUART A. AARONSON
Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20205

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Infants' Discrimination of the Duration of a Rapid Spectrum Change in Nonspeech Signals

Abstract. *Two-month-old infants discriminated complex sinusoidal patterns that varied in the duration of their initial frequency transitions. Discrimination of these nonspeech sinusoidal patterns was a function of both the duration of the transitions and the total duration of the stimulus pattern. This contextual effect was observed even though the information specifying stimulus duration occurred after the transitional information. These findings parallel those observed with infants for perception of synthetic speech stimuli. Specialized speech processing capacities are thus not required to account for infants' sensitivity to contextual effects in acoustic signals, whether speech or nonspeech.*

More than a decade of research has demonstrated infants' capacities for processing speech sound (*1*). Early beliefs that these capacities reflected innate linguistic abilities were based exclusively on findings demonstrating discrimination of various speech contrasts in an adultlike manner analogous to categorical perception (*2, 3*). At the time, such categorical discrimination effects were thought to occur only for speech stimuli. However, subsequent research with both adults and infants indicated that similar effects could be obtained with certain nonspeech signals (*4, 5*). Instead of being specific to speech processing, it seemed that the infant's speech processing could be accounted for in terms of general auditory processing capabilities (*6*).

Recently, arguments for proposing specialized innate speech processing capacities in humans have relied on phenomena such as context effects and trading relations in which perceptual boundaries for phoneme categories were shown to depend on multiple sources of information relating to factors such as speaking rate and the nature of the surrounding phonemes in the utterance (*7*). For example, Miller and Liberman (*8*) found that changes in duration related to different speaking rates affected the interpretation of the acoustic correlates of the stop-glide contrast between /ba/ and /wa/; the perceptual boundary for formant transition duration (an acoustic correlate of the stop-glide distinction) was affected by the overall duration of the stimuli (*9*). For stimuli of short durations (ones generated during rapid speech), the perceptual boundary along a continuum of formant transition durations occurred at shorter durations than when stimuli of long duration (corresponding to slower rates of speaking) were used. Miller and Liberman argued that an explanation of their results in terms of the psychophysical properties of the stimuli was inadequate, and hence, that the only possible explanation for these perceptual adjustments was one that invoked specialized speech processing capacities. This hypothesis has gained some additional support from a recent study of infants by Eimas and Miller (*10*). They reported that 2-month-old infants' discriminations of synthetic consonant-vowel syllables differing in formant transition durations were affected by the overall durations of the syllables so that pairs of short syllables were discriminated at shorter transition durations than were pairs of long stimuli. Eimas and Miller interpreted their results as evidence for the operation of context effects in infant speech perception analogous to those observed with adults (*8*). The implication is that infants are equipped with specialized speech processing capacities that permit them to respond to speech sounds in a "relational" and "nonlinear" fashion (*11*).

Although these studies suggest that both infants and adults are sensitive to factors relating to speaking rate, they do not necessarily demonstrate that the underlying perceptual mechanisms are specific to processing speech signals. Neither study tested whether similar effects might be present for the perception of nonspeech signals (*12*). Such a test is critical to an understanding of the underlying perceptual mechanisms. For example, a demonstration that nonspeech sounds are processed differently would support the notion that the observed context effects in speech perception are the result of specialized speech processing capacities. On the other hand, a demonstration of context effects for nonspeech sounds that parallels those observed for speech stimuli would contradict a specialized mode of speech processing. A more parsimonious explanation of both the speech and nonspeech results could then be framed in terms of general perceptual constraints on the human auditory system (*13*).

We sought to determine whether a general auditory processing account can account for the infant's sensitivity to changes in speaking rate. Previous research with adults by Carrell *et al.* (*14*) used nonspeech control stimuli to demonstrate effects comparable to those reported by Miller and Liberman (*8*). Perceptual judgments of the initial frequency transition durations of nonspeech stimuli varied with changes in the overall durations of the stimuli. Thus, these results are consistent with an auditory processing account of context effect in speech perception. A specialized speech processing account cannot be entirely ruled out, because it is still possible to argue that the adults' performance derived in some way from a covert labeling strategy involving specialized speech processing capacities. For example, adults might have covertly assigned verbal labels such as "ba" and "wa" to the nonspeech stimuli and then processed them using special speech processing capacities (*15*). This alternative explanation would be countered if the parallels