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Cellular Transforming Genes in Cancer*

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Genes which regulate growth in normal cells during differentiation function in response to appropriate signals which allow proliferation and maturation to proceed in an orderly manner. In the neoplastic cell, genetic control of proliferation appears to be lost. Non-neoplastic cells may contain a number of genes with potential transforming activity which are normally well regulated and only expressed at a particular phase of cell differentiation. Abnormal expression of these genes may result in neoplastic growth. Experiments by Cooper and co-workers have addressed the question of potential transforming activity of genes from normal cells [1]. In this series of experiments, DNA from normal cells was prepared, sheared to a size range of 0.5–5.0 kilobases, and transfected as a calcium phosphate precipitate into NIH 3T3 cells. Transforming efficiences of high molecular weight DNAs from these cells were approximately 3×10^{-4} transformants/μg DNA, while transforming efficiencies for sheared DNAs were tenfold greater. Transforming efficiencies of DNAs from foci isolated in primary transfection of sheared DNA when tested in secondary transfection were 100–1000 fold higher (0.1–1.0 foci/μg DNA), comparable to transforming activities of strongly oncogenic viruses [2, 3]. These studies suggest that normal cell genes, when expressed abnormally, can transform at high efficiencies.

High molecular weight DNA from neoplastic cells, unlike that from normal cells, transforms NIH 3T3 cells in primary transfection with high efficiencies of 0.1–1.0 transformants/μg DNA, suggesting that events at the DNA level have occurred already which have freed these genes from appropriate control. Activated transforming genes which can be efficiently transmitted by transfection with high molecular weight DNAs have been found in chemically transformed mouse fibroblasts [4], B-cell lymphomas and a nephroblastoma induced by avian lymphoid leukosis viruses [5], human bladder carcinomas [6, 7], mammary tumors of mouse and human origin [8], gliomas and neuroblastomas of rat and mouse origin [9], and human colon carcinoma and promyelocytic leukemia [10]. The transforming genes detected by transfection of DNAs of tumors induced by lymphoid leukosis viruses, a class of retroviruses which lack viral transforming genes, and those of mammary tumors associated with mouse mammary tumor virus, which also lacks a viral transforming gene, are not linked to viral DNA sequences, suggesting that oncogenesis by these viruses involves indirect activation of cellular transforming genes [5, 8].

Hayward and co-workers [11] have demonstrated that in LLV-induced tumors, a cellular gene (*c-myc*), homologous to the transforming gene of acute leukemia virus, is activated by adjacent integration of viral DNA (LTR insertion). However, analysis of NIH cells transformed by DNAs of these neoplasms indicates that transformation

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was not mediated by transfer of the *c-myc* gene to the NIH cells [12]. These observations indicate that at least two different cellular genes are activated in LLV-induced neoplasms. As carcinogenesis is a multistage process, activation of *c-myc* may precede or complement activation of the cellular transforming gene detected by transfection. Alternatively, LLV may act at an early preneoplastic stage to expand the population of cells in which transforming events occur, which is consistent with the mechanism proposed by McGrath and Weissman for induction of T-cell lymphomas by analogous retroviruses of the AKR mouse [13]. These findings thus suggest that transformation by a variety of carcinogenic agents can involve dominant mutations or gene rearrangements resulting in the activation of cellular transforming genes which are then detectable by transfection.

Weinberg and co-workers have demonstrated that mouse fibroblasts transformed by several different chemical carcinogens contained related activated transforming genes as determined by restriction enzyme analysis [4]. We have studied a series of mammary tumors of mouse and human origin. In these experiments, high molecular weight DNAs of five mouse mammary tumors, two chemical carcinogen-induced mouse mammary tumors, and one human mammary tumor cell line (MCF-7) were assayed for the presence of transmissible activated transforming genes by transfection. DNAs of six mouse tumors and the human tumor cell line induced transformation with high efficiencies. The transforming activities of DNAs of all five MMTV-induced tumors, the chemical carcinogen-induced mouse tumor, and the human tumor cell line were inactivated by digestion with the restriction endonucleases *Pvu*II and *Sac*I, but not by *Bam*HI, *Eco*RI, *Hind*III, *Kpn*I, or *Xho*I. These results suggest that the same transforming gene was activated in six different mouse mammary carcinomas, induced by either MMTV or a chemical carcinogen, and in a human mammary carcinoma cell line [8]. This transforming gene differs by restriction analysis from that activated in two human bladder carcinomas [6] and from the gene activated in chemically transformed fibroblasts [4]. These findings suggest that within neoplasms of a particular differentiated cell type, a common transforming gene is activated. Cloning of the human mammary tumor transforming sequence has recently been completed. Transforming efficiency of this clone is 40,000 foci/μg cell DNA insert, and as expected from our previous studies, the transforming sequence contains both *Sac*I and *Pvu*II sites.

Sera from mice-bearing tumors induced by NIH 3T3 cells transformed by mouse or human mammary tumor DNA immunoprecipitated an 86,000-dalton glycoprotein in extracts of NIH cells transformed by human mammary carcinoma DNA. This antigen was also immunoprecipitated by sera from mice-bearing tumors induced by mouse mammary carcinoma DNAs and from mice-bearing primary mammary carcinomas. These results indicate that this protein represents an antigen which is specifically associated with expression of the transmissible transforming genes of human and mouse mammary carcinomas [14].

Molecular cloning of the transforming gene activated in chicken bursal lymphoma has been carried out by Goubin et al. [15]. Use of this cloned gene as a probe in Southern blotting analysis indicates that this gene is evolutionarily well conserved in that hybridization to human DNAs is of comparable intensity to that observed in hybridization of the probe to chicken DNA. The cloned chicken bursal gene is biologically active and transforms with efficiencies 10^5 times enriched over uncloned bursal tumor cell DNA.

There are several conclusions to be drawn from these studies. We may first assume that cellular transforming genes, in their normal state, are carefully regulated and may only be expressed or "turned on" at a particular phase of cellular differentiation in response to the appropriate external signals. We can speculate that, as a rare event, a dominant genetic change in the control elements of these genes can lead to unregulated growth which results in the production of a neoplasm. Clearly, one or several steps may be involved in the deregulation of such a gene. The end result, however, may be successfully scored by transfection. A second point which emerges is that these genes appear to be well con-

served in evolution, based upon the apparent similarity by restriction enzyme analysis between human and mouse mammary tumor transforming genes [8, 14], and from results obtained with the cloned chicken bursal transforming gene [15]. This suggests that a gene cloned from a neoplasm of mice may share substantial homology with the analogous gene in humans. These studies also demonstrate that within a particular differentiated phenotype, a common transforming gene is activated which may be particular to the differentiated state of the neoplasm.

Considering these data, we have speculated that in a specific cell lineage which undergoes several differentiative steps to maturity, one or several of the genes expressed at these steps may be susceptible to rearrangements or mutations which can lead to neoplastic transformation. To examine this hypothesis more closely, we have chosen a cell lineage where differentiation can be described in terms of migration, surface markers, and cellular function in maturity. Cells of the immune system seem most appropriate for these investigations.

Within the immune system, the differentiative lineages of B- and T-lymphocytes are perhaps the most well characterized. Cell surface markers exist, which allow classification of cell types as early, intermediate, or mature in both human and murine systems. Neoplasms which arise from T- and B-lymphocyte populations have been characterized with regard to their state of differentiation by surface markers, by function in in vitro assays, and, in B-cell neoplasms, by the degree of heavy and light chain production, assembly, and secretion. These neoplasms can be categorized as representative of early, intermediate, or mature counterparts of normal T- and B-lymphocyte differentiation. Identification, characterization, and molecular cloning of cellular transforming genes from T- and B-lymphocyte neoplasms, representative of early, intermediate, and mature stages of normal cellular differentiation, will provide the tools to examine genetic events which lead to neoplastic transformation. These studies will allow us to assess the number of genes at risk of neoplastic transformation within a well-described normal cell differentiative lineage. Probes generated in these experiments may provide new insights into the mechanisms by which growth is regulated in normal lymphocytes and may lead to an understanding of molecular events which signal cellular proliferation and differentiation.

To determine whether transmissible transforming genes could be detected in T- and B-lymphocyte neoplasms by transfection of NIH 3T3 cells, we prepared high molecular weight DNA (> 20 kb) from more than 20 different neoplastic cell lines or primary patient isolates from T- and B-lymphocyte neoplasms of mouse or human origin. Calcium phosphate precipitates of these DNAs were applied to NIH 3T3 cells, and primary transformation efficiencies were scored after 12–14 days of culture [16]. Efficiencies of these DNAs in primary transfection ranged from 0.05 to 0.5 foci/μg DNA. Four to six foci from each primary transfection were picked and grown in mass culture. DNAs were prepared from these foci of NIH cells transformed by tumor DNAs and were used as donor DNAs in secondary transfection assays. Transforming efficiencies in secondary transfection also ranged between 0.05 and 0.5 foci/μg DNA, indicating that transmissible activated transforming genes from the neoplasms were present in the transformed NIH cells. DNA from spontaneous transformants, which occasionally arise in NIH 3T3 cells, does not retransform in secondary transfection assays; thus we were assured that these assays were identifiying only dominant activated transmissible transforming genes in transformants from these neoplasms. Transforming efficiencies for some of the T- and B-neoplasm DNAs tested in primary and secondary transfection assays are presented in Tables 1 and 2. Efficient transformation of NIH cells were achieved with DNAs from frozen patient cells, primary tumors, or cell lines of both mouse and human origin. From this table it may be also observed that from B-lymphocyte lineage neoplasms we have obtained transformation using DNA from tumors representative of early, intermediate, and mature stages of normal differentiation. From neoplasms of the T-lymphocyte lineage we have identified transformants representative of intermediate

Pre-T (?) neoplasm	Intermediate T neoplasm	Mature T neoplasm
Human None *Mouse* (2) SJL Transforming efficiency = 0.15 foci/μg DNA	*Human* (1) T10[a] *Mouse* (1) S49 (1) W7.1 (1) L691 (1) KKT2 (1) SL3 (1) SL7 Transforming efficiency = 0.10 – 1.0 foci/μg DNA	*Human* (1) Sezary Syndrome[a] *Mouse* (1) Clone A (1) 104.6 Transforming efficiency = 0.05 – 0.55 foci/μg DNA

Controls	
Normal mouse thymocytes	< 0.003
Helper clone 101.6	< 0.001
Human embryo fibroblasts	< 0.002
Salmon sperm	< 0.005

Table 1. T-lymphocyte neoplasms possessing transmissible transforming genes

Cell lines or patient isolates designated (1) are described by Lane et al. [16]. Cell line designated (2) was obtained from M. Schied

[a] Indicates primary tumor

and mature stages of normal differentiation. Because of a lack of cell surface markers to characterize prethymic tumors, obtaining a representative for this classification has proved difficult. At present, we have several candidate neoplasms which show no heavy chain gene rearrangement, have no theta on their surface, and are terminal transferable inducible. Assays are currently in progress to determine whether DNAs from these tumors will transform NIH 3T3 cells in culture. With the completion of these studies, we will have identified transmissible transforming genes from T- and B-lymphocyte neoplasms which represent early, intermediate, and mature stages of normal differentiation, and will have completed our first goal in these studies.

Restriction enzyme analysis of transforming genes has proved useful in two ways. First, it has allowed us to demonstrate that activation of a specific transforming gene is correlated with a particular differentiated cell type in neoplasms representative of that particular stage of normal differentiation. Second, identification of restriction enzymes which do not inactivate transforming genes provides a useful cloning strategy by means of a transforming gene enrichment step.

To carry out this type of analysis, we selected four six base recognition restriction endonucleases which cleave cellular DNA statistically once every 4 kb. We chose *Eco*RI, *Hind*III, *Bam*HI, and *Xho*I to cleave whole cell DNA containing transforming genes of interest. Digestions were monitored by gel electrophoresis to assure that complete digestion had occurred. Both digested DNAs and companion undigested samples were transfected onto NIH 3T3 cells, and foci were enumerated. If a transforming gene possessed the six nucleotide base sequence recognized by the restriction endonuclease, the gene would be cleaved. Cleavage within the transforming gene sequence resulted in a reduction in transformation efficiencies by direct inactivation of the transforming gene. In this manner, a "finger print" of the transforming gene could be generated based upon enzyme inactivation patterns.

These findings are summarized in Table 3. We are currently continuing and expanding these. To date, by this method of

Table 2. B-lymphocyte neoplasms possessing transmissible transforming genes

Pre-B neoplasms	Int. B neoplasms	Mature B neoplasms
Human	*Human*	*Human*
(1) $C^+B^+1^a$	(5) Raji	(1) GM1500
(1) $C^+B^+2^a$	(5) Namalwa	(1) GM2132
(1) $C^-B^-1^a$	(5) BJAB	*Mouse*
(1) $C^-B^-2^a$	(6) MC116	(1) S107
(1) 207	(6) EW36	(1) M315
(1) 697	(6) CW678	(1) NS2.1
(2) SMS-SB	*Mouse*	Transforming efficiency 0.11 – 0.20 foci-μg DNA
(3) NALM-1	(1) W231	
Mouse	(1) 2PK3	
(4) B6T4E4	(1) BCL-1	
(4) B6T1E1	Transforming efficiency 0.15 – 1.2 foci/μg DNA	
Transforming efficiency 0.09 – 1.10 foci/μg DNA		

Controls	
EBV immortalized B-lymphocytes (2)	< 0.005
Hu emb. fibroblasts	< 0.002
Salmon sperm	< 0.005

Cell lines or patient isolates designated (1) are described by Lane et al. [16]. (2) Human pre-B cell line prepared by G. Smith and B. Ozan manuscript in preparation. (3) Blood, Vol. 58:648 (1981). (4) Mouse Ablesome-induced pre-B tumors, C57BL/6 strain from R. Risser. (5) African Burkitts patient cell lines from I. McGrath. (6) American Burkitts cell lines from I. McGrath

[a] Indicates primary tumor

Table 3. Restriction analysis of B-lymphocyte neoplasm transforming sequences

	*Eco*RI	*Bam*HI	*Xho*I	*Hind*III	*Sac*I
Pre-B neoplasms					
Six human	–	+	+	–	ND
Two mouse	–	+	+	–	ND
Intermediate B neoplasms					
Two human	–	+	–	–	ND
Three mouse	–	+	–	–	ND
Mature B neoplasms					
Two human	–	–	–	–	+
Two mouse	–	–	–	–	+

Restriction analysis of T-lymphocyte neoplasm-transforming sequences

	*Eco*RI	*Bam*HI	*Xho*I	*Hind*III
Intermediate T neoplasms				
One human	+	–	–	–
Six mouse	+	–	+/–	–
Mature T neoplasms				
One human	–	–	+	+
Two mouse	–	–	+	+

analysis, we have identified three different B-cell transforming genes and two different T-lineage transforming genes. In these groups, a common gene appears to be activated in both mouse and human neoplasms within a differentiated cell type, again suggesting the presence of evolutionarily well-conserved genes. The restriction analysis of each of these genes establishes them as distinct from transforming genes of human bladder carcinoma [6], mouse and human mammary tumors [8], and chemically transformed fibroblasts [4]. The transforming genes thus far identified correlate well with phenotypic expression within particular differentiated cell types. It is our hypothesis that these genes represent cell growth genes which have become freed of normal regulatory constraints. When cloning of a representative gene from each of the five groups is completed, we will use these genes as probes to determine what signals trigger their expression during normal cellular differentiation, and we hope to determine at which stage these genes are at risk of neoplastic changes.

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