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Retroviral and Human Cellular Oncogenes

JOSEPH G. SINKOVICS, M.D.*

St. Joseph's Hospital, Community Cancer Center, Tampa, FL 33677

ABSTRACT

Unexpected meeting of two separate lines of research resulted in the discovery of oncogenes. Oncogenes are deoxyribonucleic acid (DNA) sequences coding for polypeptide gene products which cause, or contribute to, neoplastic growth of cells. Oncogenes remain almost unchanged through evolution: oncogenes and their gene products of avian, murine, feline, simian and human species show close homology.

Retroviruses possess three genes encoding virion structural proteins and envelope. The DNA copy of the viral genome (the provirus) recombines with DNA sequences of the host cell genome and thus acquires an additional DNA sequence of host origin (transduction). The newly acquired DNA sequences render the retrovirus oncogenic.

Certain genomic DNA sequences extracted from human tumor cells induce malignant transformation in selected assay systems (transfection). The transforming genes of retroviruses show close homology to these cellular oncogenes. Retroviruses appear to have acquired cellular proto-oncogenes during past interactions with their host cells.

In the cell, proto-oncogenes are presumed to fulfill fundamental functions of cell differentiation and mitosis. This is deduced from their preservation during evolution, i.e., proto-oncogenes of avian, lower and higher mammalian and human species display close DNA sequence homology and thus their gene products are also similar in distant species. When expressed in excess or in altered form or at a wrong chromosomal location or at an inappropriate time of the cell cycle, proto-oncogenes function as oncogenes by inducing mitoses and inhibiting differentiation of their host cells.

The Retroviral Genome

The first line of research concerned the oncogenes of retroviruses. In the mid-1960s, it had already been surmised that the retroviral genome could code for the replication of the virion without causing malignant transformation of the host cell. Our own encounter with this phenomenon occurred in connection with the development of an attenuated mouse leukemia virus strain. Tissue cultures infected with a mouse leukemia virus either underwent malignant transformation[52] or produced type C virus particles in abundance.[50] Large in-

* Visiting professor, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, TX.

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TABLE I

Immunization with and Reactivation of Attenuated Mouse Leukemia Virus

Inocula (dilutions)	*Days between inocula*	*Percent leukemia*	*Duration of life (days) with leukemia*
AV cc VV $10^{-1.7}$	12	10-38	166-176
AV cc VV $10^{-3.7}$	1	100	55- 64
AV cc ---------	--	8	>176
----- VV $10^{-1.7}$	--	100	52
----- VV $10^{-3.7}$	--	22	102

AV = attenuated virus
cc = concentrated (undiluted tissue culture fluid)
VV = virulent (mouse passage) virus
Different experiments of the same series were reported in 1966.[49,50]

ocula of these virus particles induced no or very low incidence of leukemia in mice. The live avirulent virus immunized mice against the leukemogenic mouse passage line virus when the attenuated virus was given as an undiluted inoculum more than 10 days before the inoculation of a low dilution ($10^{-1.7}$) of the leukemogenic virus (table I). The unexpected finding emerged when undiluted inocula of attenuated live virus were given one day before high dilutions ($10^{-3.7}$) of the leukemogenic virus. In this latter circumstance, instead of protection against leukemogenesis as expected, a high incidence of leukemia followed. The phenomenon resembled "multiplicity reactivation" of partially inactivated viruses. It was immediately suggested that highly diluted leukemogenic virus replicated its leukemogenic genome fragment in excess of the virions that could have incorporated all these fragments. It was postulated that replicating attenuated virus particles incorporated leukemogenic genome fragments resulting in the restoration of their virulence (i.e., leukemogenic potency). "Nonleukemogenic virus particles probably are able to utilize fragments of the genome of leukemogenic particles; this part of the viral genome is probably reproduced in excess during ordinary reproduction of leukemogenic virus particles. Thus, in an animal inoculated with the proper proportion and sequence of large doses of nonleukemogenic, and small doses of leukemogenic virus particles, accelerated leukemia attributable to reactivation of the nonleukemogenic virus particles may occur."[49]

Observations like this and those of more sophistication formed the emergence of retrovirus genetics.[62] These more advanced observations led to the formulation of the virogene-oncogene hypothesis.[20,59] According to this theory, the retroviral genome consists of genes referred to as virogenes encoding the structural proteins, reverse transcriptase and envelope of the virions. In addition, acutely transforming retroviruses possess another gene responsible for the malignant transformation of the host cell: the oncogene.

The first retroviral genome analyzed thoroughly is that of the Rous sarcoma virus. The genome of this avian sarcoma virus consists of genes *gag*, *pol*, *env*, and *src*. The first three genes code for the synthesis of internal structural proteins, reverse transcriptase (polymerase) and envelope proteins of the virion. The v-*src* (sarcoma) gene encodes the synthesis of a phosphokinase. This enzyme is a tyrosine-specific kinase as it attaches a phosphate group to tyrosine, whereas most other kinases phosphorylate serine. It is through the actions of this enzyme that the cells assume neoplastic behavior. The v-*src* gene is fully oncogenic in itself both in 3T3 mouse fibroblasts in culture or *in vivo* in young chickens.[14,22,58]

Transfection

The second line of experimental work concerned DNA strands extracted from human tumor cells. These DNA sequences are capable of causing malignant transformation *in vitro* in an assay system of NIH3T3 mouse fibroblasts.[27]

TABLE II

Detection of New Antigen Expression in Human Embryonic Fibroblasts Inoculated with Cell-free Fluid from Cultured Sarcoma Cells: Antigenic Conversion

Positive Immunofluorescence for New Antigen / *Number of Tests*	*Percent Positive*	*Percent Range*
51/336	15.6	9.7-60

These experiments were reported from 1971 to 1977.[53,54,55]

More than a decade earlier, attempts were made at the transfer of human tumors by means of cell-free filtrates to animals or to tissue culture systems; however, none of these attempts yielded conclusive results. The most successful of these attempts was that of Morton and associates demonstrating antigenic conversion and cell focus formation in cultures of human embryonic cells inoculated with cell-free fluids derived from human sarcomas and their cultured cells.[35] These experiments were immediately confirmed by Giraldo and associates[16] and by the author of this review[53,54,55] (table II) because this very attractive line of research was apparently pursued contemporaneously in several laboratories. However, the nature of the transforming principle has never been determined. A human sarcoma-inducing retrovirus was repeatedly suggested for this role but no such virus could ever be isolated and identified.

The modern transfection experiments yielding the first isolated and cloned human oncogenes are far more sophisticated than these early attempts. The genomic DNA derived from carcinoma cells of the urinary bladder is cleaved by restriction endonucleases into DNA sequences. The purified high molecular weight DNA is precipitated in calcium phosphate and incubated with recipient cells under the conditions of glycerol shock to facilitate uptake of the exogenous DNA. The calcium phosphate-DNA matrix is phagocytized by recipient cells of the 3T3 assay system; DNA transferred into the nucleus is incorporated in the genome of the recipient cells. When the incorporated DNA is an oncogene, the recipient cells begin to grow in a contact-uninhibited fashion forming foci of rounded up cells. The human oncogene can be identified in and extracted from the transformed cells and used to reinduce neoplastic transformation in newly inoculated 3T3 fibroblasts. The calcium phosphate technique yielded cell oncogenes from large numbers of human tumors including both carcinomas and sarcomas and leukemias.[27] The oncogene can be re-extracted from the transformed cells and the transformed cells grow as tumors in athymic nude mice subcutaneously or in the lungs after intravenous inoculation.

Transduction

The two lines of research, the first recognizing the retroviral oncogenes and the second resulting in the isolation and cloning of cellular oncogenes, met when the homology of retroviral and cellular oncogenes has been recognized.[10,63]

For example, the *myc* oncogene of the avian myelocytomatosis virus (v-*myc*) is present in the genome of chicken, murine, and human cells either as a proto-oncogene in normal cells or as an amplified oncogene in neoplastic cells. Another example is the group of *ras* oncogenes. These oncogenes were extracted from a large number of human tumors and also serve as the oncogenes of rat sarcoma viruses of Harvey and Kirsten (H- or K-ras for rat sarcoma).

The best explanation for this overlap of homology between retroviral and cellular oncogenes is that retroviruses once existing with a virogenic genome only have acquired proto-oncogenes from host cells through recombinations of the DNA

copy of the viral genome (the provirus) with DNA sequences of the host cell genome. These host sequences are those preserved in unusually stable (unchanged) form throughout evolution expressing great similarities in distant species, i.e., close homology in avian, murine, feline, simian and human species. From the stability of these DNA sequences maintained unchanged during evolution, it is deduced that the so-called acutely transforming retroviruses have acquired their oncogenes from their host cells through genetic recombination with host genes (transduction).

Oncogenes

Oncogene *src*

The cellular progenitor of v-*src* is proto-oncogene c-*src*. Avian retrovirus strains devoid of v-*src* gene can acquire c-*src* through infection of avian cells. The DNA copy of the viral genome (the provirus) undergoes recombination with the cellular DNA containing the v-*src* segment. Transcription of the hybrid DNA into chimeric ribonucleic (RNA) follows; virions contain heterozygous viral RNA. Subsequent infection with such virions results in recombination with host cell DNA but the viral genome already includes the cellular gene as its own.[58]

The c-*src* gene of normal cells differs slightly from v-*src* or from c-*src* extracted from tumor cells. The c-*src* of chicken and human cells differ slightly. The preservation of proto-oncogene throughout evolution suggests that these genes fulfill basic function in cell division and differentiation.

The location of human c-*src* is in chromosome 20. When transformation-defective avian retroviruses pass through human cells, they may pick up c-*src* and become transforming retroviruses.

The gene product of *src* is a tyrosine kinase referred to as pp60*src*.[22]

Oncogene *myc*

Avian myelocytomatosis virus strain MC-29 carries oncogene v-*myc*. Avian cellular oncogene c-*myc* was presumably picked up by ancestral retrovirus infecting birds. Possession of c-*myc* (now v-*myc*) rendered this virus strain highly oncogenic in chicken causing myeloid leukemia, renal and hepatic carcinoma; sarcoma.

The avian leukosis virus is devoid of an oncogene of its own. It causes B cell lymphoma of the bursa in chickens by inserting a DNA copy of the viral RNA genome next to c-*myc* resulting over 30-fold overproduction of c-*myc*, its mRNA and gene product, a double stranded DNA-binding protein.

The normal location of human c-*myc* is in band 24 on the long arm of chromosome 8 (8q24). Human c-*myc* is not detectable by transfection experiments in 3T3 cells but v-*myc* of the avian myelocytomatosis virus MC-29 induces foci of transformed cells in transfection experiments of 3T3 cells.

A recent isolate of cytomegalovirus (a virus implicated in the causation of immunosuppression in acquired immunodeficiency syndrome and Kaposi's sarcoma) contains genome sequences homologous to v-*myc*.[56]

In Burkitt's lymphoma cells reciprocal translocation between chromosomes 8 and 14 takes place: 8q24 moves to chromosome 14q32 and 14q32 to 8q24. Less frequent translocations move c-*myc* to 2p11 or to 22q11. The translocated c-*myc* is situated within regions where genes encoding Ig heavy and light chains (kappa or lambda) reside. This translocation of c-*myc* is independent from the presence or absence of Epstein-Barr virus (EBV); B lymphocytes immortal-

ized by EBV do not undergo c-*myc* translocations. Translocated c-*myc* escapes normal transcriptional control to which the untranslocated c-*myc* gene is subjected. High levels of the gene product protein turn off c-*myc* in its normal location but translocated c-*myc* fails to respond to normal regulatory mechanisms.[24,31,32,34,38] Translocated c-*myc* may come under the transcriptional control of the immunoglobulin genes.

Amplification of c-*myc* occurs in human acute promyelocytic leukemia, colonic carcinoid, neuroblastoma (N-*myc*) and small (oat) cell carcinoma variants; c-*myc* was amplified in cell lines of human T cell leukemia.[28,29]

Oncogene *abl*

The Abelson murine leukemia virus harbors v-*abl* as its transforming gene; this virus is considered to be the Moloney mouse leukemia virus recombined with a c-*abl* oncogene.

Normal location of human c-*abl* is in band 34 on the long arm of chromosome 9 (9q34). The Philadelphia chromosome in chronic myelogenous leukemia is the result of a balanced reciprocal translocation t9q34:22q11 (9:22;24:11) in which oncogene c-*abl* moves from its normal position to band 11 on the long arm of chromosome 22 (22q11), where normally the lambda light chain gene resides. In turn, oncogene c-*sis* translocates from its normal position on chromosome 22 to the long arm of chromosome 9.[2,5,19]

Oncogene *sis*

The simian (woolly monkey) sarcoma virus causes fibrosarcomas through the actions of its oncogene v-*sis*. The cellular counterpart of this oncogene is c-*sis* which encodes as its gene product platelet-derived growth factor. The two oncogenes v-*sis* and c-*sis* have close DNA homology and their gene products have nearly identical amino acid sequences.

Human oncogene c-*sis* resides in band 13 on the long arm of chromosome 22 (22q13). Its gene product p28, a 226 amino acid protein known as platelet-derived growth factor (PDGF) mediates a number of biologic functions: it is mitogenic to mesenchymal and glial cells; induces fibroblast proliferation in wound healing; induces smooth muscle and fibroblastic proliferation in arterial walls when platelets attach to arteriosclerotic plaques. Its presumed pathological functions are induction of fibroblast proliferation in myelofibrosis (a fatal myeloproliferative syndrome frequently associated with thrombocytosis) and in sarcomagenesis.[7,8,21,57]

Occasionally one of the feline sarcoma viruses carries either the v-*sis* or the v-*abl* oncogene.

Translocation of human c-*sis* occurs in Philadelphia chromosome-positive chronic myelogenous leukemia (see oncogene *abl*).[2]

A chromosomal translocation at band q12 of chromosome 22 has been observed in Ewing's sarcoma. This observation implied translocation and amplification of the c-*sis* oncogene in this tumor. However, no activation of the c-*sis* oncogene could be documented in Ewing's sarcoma cells.[3] The c-*sis* oncogene was found in activated or amplified state in several human tumors.

Activation of c-*sis* was detected in human T cell leukemia virus-induced neoplastic T cells but c-*sis* mRNA was not regularly expressed in these cells.

Oncogene *ras*

Functions as the transforming oncogene of Harvey and Kirsten rat sarcoma, and Balb mouse sarcoma, viruses (v-*ras*), known as *has*, *kis*, and *bas*. The Harvey rat sarcoma virus is the result of recombination between the Moloney mouse

leukemia virus and rat c-*ras* gene. It was discovered by transfection technique as active oncogene in large numbers of human tumors (c-*ras*). Of human carcinomas (lung, in particular small cell undifferentiated, bladder, breast, colon, gallbladder, pancreas), 27 percent yielded transforming oncogenes. Squamous cell carcinomas of uterine cervix, head and neck and esophagus and adenocarcinomas of kidney and ovary seldom if ever yielded active oncogenes in transfection experiments in the 3T3 assay system. Of mesenchymal tumors, 50 percent of leukemias and lymphomas and sarcomas (osteosarcoma, rhabdomyosarcoma) contained extractable oncogenes. Of neurogenic and neuroectodermal tumors (glioblastomas, astrocytomas, melanomas and neuroblastomas), 21 percent yielded transforming oncogenes.[27] These tumors contained either the H-*ras* (bladder carcinoma) or the K-*ras* (colon, pancreas and lung carcinomas; mesenchymal tumors) or the N-*ras* (neuroblastoma; mesenchymal tumors) oncogenes.

Human H-*ras* and K-*ras* oncogenes reside on the short arm of chromosome 11 and 12, respectively; N-*ras* was mapped to chromosome 1p21.[42]

The gene product of *ras* oncogenes is a polypeptide with ability to bind to guanoside (guanine nucleotides).

Proto-oncogene and oncogene *ras* in one bladder carcinoma cell line differ only at a single point reflected as replacement of one amino acid in the gene product (table III).

The proto-oncogene may undergo "spontaneous" change without interaction with known carcinogenic agent: A deoxyguanosine located at position 35 of the first exon of the c-*has/bas* proto-oncogene was changed into a deoxyadenosine in the oncogene. This oncogene is expected to direct the incorporation of aspartic acid as the 12th amino acid of the gene product p21 protein.[11,45,48] Another gene product consequential to mutation in codon 61 of H-*ras* oncogene in lung carcinoma contained leucine instead of glutamine. Any point mutation altering the coding function of codon 12 or codon 61 of *ras* proto-oncogenes will result in the acquisition of transforming potency.

TABLE III

One Point Mutation between H-ras Proto-oncogene and Oncogene

Sites changed	*Proto-oncogene*	*Oncogene*
1st exon, 35th position	Guanosine-guanosine-cytosine	Guanosine-thymidine-cytosine
12th amino acid of p21 protein	Glycine	Valine

For genomic nucleotide sequences and translation, see J. Am. Med. Assoc. 248:2418-2426, 1982.

While pancreatic adenocarcinoma cells, ovarian cystadenocarcinoma cells and squamous lung carcinoma cells contained activated K-*ras* oncogenes, normal cells of these tumor-bearing patients did not contain activated K-*ras* oncogenes.[12,44] However in normal regenerating liver,[18] expression of c-*ras* is temporarily increased.

Other Oncogenes

Oncogene *mos* serves as the transforming gene of Moloney mouse sarcoma virus (v-*mos*). The gene product is a 42000 MW protein functioning as a tyrosine kinase. Isolated v-*mos* is not active in transfection assay. Long terminal repeat sequence of the viral genome together with v-*mos* induce malignant transformation.[25,28,29] Human c-*mos* is located in band 22 of the long arm of chromosome 8 (8q22). Translocation of c-*mos* occurs from chromosome 8 to 21 in acute myelogenous leukemia: t(8:21). Activation of c-*mos* occurs in Burkitt's lymphoma.

Oncogene *myb* functions as the transforming oncogene of avian myeloblastosis

virus. Human myeloblastic leukemia cells express activated homologue (c-*myb*) of the v-*myb* oncogene. When these cells were induced to differentiate by 12-0-tetradecanoylphorbol-13-acetate, the level of c-*myb* expression rapidly declined.[6] Expression of c-*myc* and c-*myb* decreases in human promyelocytic leukemia cells when cell differentiation is induced by dimethyl sulfoxide or retinoic acid.

Oncogene c-*Blym* was detected by transfection assay from Burkitt's lymphoma cells. The human c-*Blym* oncogene is homologous to the transforming gene of chicken bursal B cell lymphoma. This oncogene has not as yet been detected in retroviruses. Human *Blym*-1 resides in band 32 on the short arm of chromosome 1 (1p32). The activation of c-*myc* and *Blym*-1 in Burkitt's lymphoma is not related to the physical proximity of these two oncogenes. Location of *Blym*-1 is also distinct from the location of N-*ras* which occupies a site between the centromere of chromosome 1 in band 21.[35]

The gene product of *Blym*-1 is a 65 amino acid protein closely homologous to transferrin. Receptor for transferrin is encoded by a gene located at 3q23; the transferrin receptor is considered to be the target structure for natural killer lymphocytes.[61] The p97 melanoma surface antigen also belongs to the transferrin family of proteins. These iron-binding proteins act as growth factors and mitogens for lymphoid cells.

Blym is activated in Burkitt's lymphoma and hairy cell leukemia (leukemic reticuloendotheliosis).[9] The neoplastic cell of this latter condition frequently displays features of an unusual B lymphocyte harboring Epstein-Barr virus[51] and patients develop unusually high titers of antibodies directed to this virus and virally encoded cellular antigens.[43]

Oncogene *fos* functions as transforming oncogene of Finkel-Biskis-Jinkins mouse osteosarcoma virus (v-*fos*). When the sequence of mouse c-*fos* is compared with human c-*fos*, a 90 percent homology is observed. Transcripts of this gene occur in human placenta and fetal tissues.[60]

Oncogenes *raf* and *mil* represent the identical or closely related transforming gene of a murine and an avian retrovirus. The avian retrovirus strain MH2 harboring v-*mil* causes hepatocellular and renal carcinomas. Two human genes are closely related to v-*raf* and are located on chromosomes 3 and 4. Human tumors showing translocations and rearrangements of these chromosomes may contain either activated (amplified) or lost c-*raf* sequences. In familial kidney carcinoma translocations involving chromosome 3p21 and 3p13-14, in small cell carcinomas of lung deletion involving 3p14, and in parotid gland tumors, translocation at 3p21 and 3p25 are known to occur.[4]

Oncogene *fes* serves as transforming oncogene of feline sarcoma virus (v-*fes*) encoding tyrosine-specific protein kinase. The Gardner-Rasheed feline sarcoma virus arose by recombination of the feline leukemia virus and host cell genomic DNA sequences (c-*fgr*). Cells transformed by this feline sarcoma virus produce a hybrid protein containing a portion of actin and a tyrosine-specific protein kinase.[37]

The human c-*fes* oncogene has been mapped to chromosome 15 and is probably translocated to chromosome 17 (t $15q^+;17q^-$) in acute promyelocytic leukemia.

This translocation consists of a reciprocal exchange of the distal segments of the long arms of these two chromosomes. The exact sites of break points of these chromosomes are not known but were tentatively placed at 15q22-23 and 17q12 or 17q21.[47]

Other oncogenes discovered in retroviruses but not investigated well enough to determine their position in the human

chromosome are *fgr* of the Gardner-Rasheed strain of feline sarcoma virus; *fms* of the McDonough feline sarcoma virus; *erbA* and *erbB* of the avian erythroblastosis virus. The *erbB* oncogene is probably derived from the proto-oncogene coding for the receptor of the epidermal growth factor; this receptor may act as a kinase phosphorylating tyrosine and once switched on cannot be easily switched off owing to loss of normal control. Further oncogenes are *ets* of E26 chicken virus; *fps* of Fujinami chicken sarcoma virus; *mos* of avian myeloblastosis virus; *rel* of the reticuloendotheliosis virus of turkeys; *ros* of the URII avian sarcoma virus; *ski* of the SK770 chicken virus; and *yes* of the Y73 chicken sarcoma virus.

TABLE IV

Translocations Involving Human Oncogenes

Neoplasm	*Gene*	*Location*	*Translocation*
Burkitt's lymphoma	Ig heavy chain	14q32	to 8q24
	kappa light chain	2p11-12	
	lambda light chain	22q11-12	
	c-myc	8q24	to 14q32; to 2p11; to 22q11
	Blym	1	
	c-mos	8	
Chronic myelogenous leukemia	c-abl	9q34	to 22q11
	c-sis	22q13	to 9q34
Acute myelogenous leukemia	c-mos	8q22	to 21
Acute promyelocytic leukemia	c-fes	15q22-23	to 17q12 or 17q21

Actions of Oncogenes

Presumed modes of oncogene activation concern *amplification*, i.e., increased levels of expression of the activated proto-oncogene consequentially to deregulation. A proto-oncogene may become deregulated when it is moved from its normal position to a new position[24] or when a retroviral genome (DNA copy of the virogene) inserts itself in the cellular genome next to a proto-oncogene. Thus, a retrovirus not possessing an oncogene of its own can induce malignant transformation by deregulating a c-*onc*.

Examples of c-*onc* amplification owing to *translocation* are shown in table IV.

The human T cell leukemia virus (HTLV) serves as an example of oncogenesis by a retrovirus devoid of its own oncogene. This exogenous retrovirus preferably infects T lymphocytes and elicits antiviral antibody production in exposed individuals. Its structural genes gag, pol and env encode virion, polymerase and envelope proteins; its long terminal repeat (LTR) sequence could activate host cell genes. The pX sequence in the genome of HTLV may function as an oncogene; it displays close homology in different isolates (from mycosis fungoides, T cell leukemic reticuloendotheliosis and acquired immunodeficiency syndrome) of the virus, whereas structural genes of different isolates mismatch.* Among the host genes specifically activated by HTLV is HT-3. If the provirus of HTLV integrates in the vicinity of genes encoding T cell growth factor (interleukin-2) and/or receptor for T cell growth factor, the infected cells will gain growth advantage over other cells. If both TCGF and its receptor are overproduced in the infected cells because of amplifications of these genes in the vicinity of HTLV provirus, the amplified genes function as oncogenes.

The structural gene of TCGF is located on the long arm of chromosome 4 (4q26-28); and the receptor for TCGF is associated with the Tac surface antigen detectable by monoclonal antibody.[33,40] Thus, leukemogenesis by HTLV may be

* Shaw, G. M., Gonda, M. A., Flickinger, G. H., Hahn, B. H., and Gallo, R. C.: Divergent human T cell leukemia virus isolates from adult T cell leukemia, hairy cell leukemia and AIDS contain a unique genomic sequence (pX) which is highly conserved. Proc. 75th Ann. Meeting American Association for Cancer Research May 9–12 Toronto, Canada. Volume 25:388, 1984, abstract #1534.

similar to avian leukosis virus which is devoid of its own oncogen but activates c-*myc* and *Blym* by promoter insertion (LTR of viral genome) in the host cell genome.

Another mode of proto-oncogene activation is through *one-point mutation* as best documented in the family of *ras* oncogenes (table III).

Combined effect of more than one oncogenes interacting might be required for multistep oncogenesis. For example, in Burkitt's lymphoma, step one is the infection of B lymphocytes by Epstein-Barr virus (EBV) resulting in immortalization but no oncogene activation. Translocations of chromosomal segments bring about activation of c-*myc* and *Blym* oncogenes. Unless a way is found to deactivate these oncogenes or neutralize their gene products, the cells remain permanently neoplastic.

If one of the basic functions of proto-oncogenes in normal cells is differentiation induction, *loss (deletion) of chromosomal segments* containing these proto-oncogenes may leave the cell permanently undifferentiated. For example, the gene encoding nerve growth factor is mapped to chromosome 1p22. This substance mediates maturation of neuroblasts to ganglion cells. Deletion of chromosomal segment 1p22 is detectable in some neuroblastomas. Deletion of 6q21 occurs in some cases of acute lymphoblastic leukemia; this chromosomal segment contains the proto-oncogene c-*myc*. H-*ras* oncogene is deleted in 11p13 in nephroblastoma (Wilms' tumor) and not clearly defined genes are ablated in 13q14 in retinoblastoma.

Oncogenes acting alone seldom can transform entirely normal cells. Continuously growing "immortalized" cells can be transformed by an oncogene acting singly. Cells not transformed by oncogene *ras* alone will be transformed by *myc* and *ras* acting together.

Retroviral (or cellular) oncogenes can interact with DNA viral oncogenes as well. For example the *ras* oncogene and adenoviral oncogene *E1A* potentiate each other.

Some DNA viruses possess transforming genes of their own (herpes, adeno- and polyoma-papova viruses) but these oncogenes are not homologous to retroviral or cellular oncogenes.

There is a locus in the human genome that shows some homology to gene enhancer sequences found in the genome of the human papovavirus BK. Thus, even some enhancer DNA sequences in the genome of oncogenic DNA viruses can be evolutionarily related to host cell sequences.[41] Oncogenic DNA virus-infected cells also release molecular mediators; example: growth stimulating factor released from herpes simplex virus type 2 infected cells.[39]

Gene Products

Activated oncogenes exert their effect through their gene products released in excess or at the wrong time in the cell cycle. In the multistep process of carcinogenesis, products of genes not necessarily recognized as oncogenes may participate. In table V are listed major growth factors.[1,13,17,62] One may postulate that overproduction of platelet derived growth factor, angiogenesis factor, certain lymphokines (TCGF or interleukin-2) and transferrin contribute to the neoplastic growth of those cells that normally respond to these mediators: mesenchymal cells (sarcomagenesis including induction of Kaposi sarcoma), T cell lymphoma and B cell lymphoma. On the contrary, lack of epidermal or nerve growth factors, owing to deletion of the corresponding genes, may prevent differentiation of epithelial cells or neuroblasts.

The gene encoding glutamate pyruvate transaminase (GPT) has been mapped to human chromosome 8 where

TABLE V

Growth Factors (GF)

Gene Product Molecular Mediator	*Effects*
Epidermal GF	Proliferation of fibroblasts. Maturation of epithelial cells.
Nerve GF	Proliferation of sympathetic ganglia. Maturation of neuroblast to ganglion cell. Differentiation of pheochromocytoma. Amino acid homology to urogastrone.
Platelet derived GF	Mitosis of mesenchymal and glial cells. Promotion of wound healing. Proliferation of leiomyoblasts in atherosclerotic plaques. Myelofibrosis in agnogenic myeloid metaplasia. Oncogenesis.
Colony stimulating GF	Monocyte and macrophage differentiation.
Erythropoietin	Induction of erythrocyte colonies and differentiation.
Angioneogenesis	Proliferation of vascular endothel.
Lymphokines	B and T cell maturation; immunoregulation.
Transferrin	Mitogenic for B lymphocytes. Its receptor is target for NK cells.
Sarcoma GF	Component α competes with epidermal GF. Components α and β exert colony forming activity and confer transformed phenotype (anchorage-independent growth) to mesenchymal cells.

also c-*myc* (8q24) and c-*mos* (8q22) reside. The GPT gene is linked to a dominant allele increasing susceptibility to breast carcinoma. However amplification of c-*myc* occurred only exceptionally in established cell lines of breast carcinoma.[23,26]

Therapeutic Implications

It is possible that interferons and other lymphokines suppress the expression of certain oncogenes.[30] Interferons induce remissions in lymphoproliferative or myeloproliferative diseases but fail to induce remissions of colonic, bronchogenic and other carcinomas. If remission induction is due to oncogene deactivation, c-*myc* and *Blym*, c-*abl* and c-*sis* oncogenes may be susceptible to interferons, whereas the *ras* oncogenes may be resistant to this effect. Resistance to interferon may develop *in vitro* (Burkitt's lymphoma cells) and *in vivo* (relapses of lymphomas after remission).

Monoclonal antibodies (YAG-172) reacting with gene product p21 proteins of H-*ras* and K-*ras* exist.[15,46] Further monoclonal antibodies could be developed to neutralize gene products released excessively or inappropriately. Elimination of these molecular mediators may result in cessation of uncontrolled growth of cells responsive to these mediators. On the other hand these mediators act as physiologic inhibitors of activated proto-oncogenes and their elimination removes control over these genes. Thus, at the present time recognition and avoidance of stimuli known to activate and amplify oncogenes are the available measures to reduce the risk of oncogenesis.

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