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Invited Review

Oncogenes and Malignant Transformation*

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Introduction – Oncogenic Retroviruses

A tumor stems from the derailment of a single cell. Not unreasonably, it has been assumed that such a dysfunction is caused by somatic mutations inactivating one or more of the finely tuned regulatory systems that govern cellular systems. In recent years, however, it has become evident that in some cases the cancerous state is not due to inactivation but to mutational activation of genes producing dominant cancer genes.

This insight was gained from experiments in which DNA from tumor material was transferred (transfected) into cultured animal cells. In some instances transfer of tumor DNA led to the formation of colonies of oncogenic cells, whereas the DNA from normal cells had no effect. With the recently developed recombinant-DNA techniques, it has been possible to isolate from the multitude of genes present in tumor cells those that were responsible for this transforming activity. Often, these genes have been found to be identical or related to the cellular homologues of the oncogenes identified in oncogenic retroviruses.

Retroviruses derive their generic name because their reproductive cycle, involves the conversion of the ss-RNA genome to a ds-DNA copy, i.e. a reversal of the classical DNA-RNA-protein sequence. This DNA copy is integrated into the host cell genome where it functions as a template for progeny virus RNA molecules. On the basis of their oncogenicity, retroviruses are classified into two groups:

a) slow leukemia viruses inducing leukemias or lymphomas after a long latency period (ca. 6 months or longer);

b) acute leukemia and sarcoma viruses inducing malignancies after a much shorter latency period.

The genome of the *slow leukemia viruses* consists of three genes designated gag, pol and env. The pol gene codes for the enzyme reverse transcriptase, whereas gag and env encode viral structural proteins. However, none of these is an oncogene in the strict sense of the word. The termini of the genome are formed by identical stretches termed Long Terminal Repeats (LTRs) on which the regulatory signals for viral gene expression are located. In several instances, the oncogenic nature of the slow leukemia viruses was found not to be based on the properties of the viral gene products, but on the regulatory activity of the LTRs: when the ds-DNA copy of the retroviral genome integrates in the vicinity of a cellular proto-oncogene (discussed below), the regulatory signals in an LTR may take control of that cellular gene and thus cause the cell to be transformed.

Nucleic acid sequence studies have shown that the *acute leukemia viruses* originally derive from the above-mentioned slow leukemia viruses. However, they also contain sequences unrelated to any of the known gag, pol and env genes. These non-viral sequences can be found at random positions of the slow leukemia virus genome where they supplant parts of the original viral genome. Studies with acute RNA tumor virus mutants have shown that the oncogenic properties of these viruses reside in the inserted sequences, which therefore were called *oncogenes*.

Further studies brought to light that the oncogenes actually are derived from the genome of the host organism and must have been acquired during a cycle of viral reproduction. E.g., v-src, the oncogene of the Rous Sarcoma Virus (RSV) has a cellular homologue in chicken (the natural host) cells, but also in other avian and in mammalian cells, and even in the cells of invertebrates. It was found to be normally expressed in noncancerous tissues. Apparently, src is a highly conserved gene which fulfills an essential function. *Mutatis mutandis*, the same can be said for other viral oncogenes: they are found universally in vertebrate and some-

Abbreviations: EGF: epidermal growth factor; PDGF: platelet-derived growth factor; LTR: long terminal repeat (of retroviral genome); RSV: Rous sarcoma virus; chr.: chromosome; Ig^H: immunoglobulin heavy chain; NMU: nitrosomethylurea; DMN: demethylnitrosamine

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Table 1. Some cellular proto-oncogenes, their possible functions, and retroviruses carrying their homologues

Function	Proto-oncogene	Virus
GTP-binding, GTPase signal transduction (membrane-associated)	c-H-ras	Harvey murine sarcoma virus (rat)
	c-K-ras	Kirsten murine sarcoma virus (rat)
	c-N-ras	no virus known
Tyrosine kinase (membrane-associated)	c-src	Rous sarcoma virus (RSV, chicken)
	c-abl	Abelson murine leukemia virus (mouse)
Growth factors and their receptors	c-sis	subunit of platelet-derived growth factor
	c-erb-B	receptor for epidermal growth factor
Control of cell proliferation (nuclear)	myc	Avian myelocytomatosis virus (chicken)
	fos	FBJ murine osteosarcoma virus (mouse)

times invertebrate species (even yeast) and are generally active in normal cells. In Table 1 some properties of oncogenes have been summarized.

Viral Oncogenes and Malignant Transformation

Since "oncogenes" were expressed in normal cells, why should they become carcinogenic once they form part of a viral genome? Several answers have been found to this question. First, the v-src gene was found to be expressed much more actively in RSV-transformed cells, than the c-src gene in normal cells. So, in this case the high level of expression of a proto-oncogene apparently could turn a normal cell into a tumor cell. Whether this interpretation was correct, was further tested by transferring other cellular proto-oncogenes to non-transformed cells. By recombinant-DNA techniques these genes were supplied either with their own regulatory signals (promoter) or with more potent promoters from other sources. In this way it was found that the cellular c-Ha-ras gene, when coupled to its own promoter, did not transform cells. When, on the other hand, it was under the control of a viral LTR (which features a strong promoter) it caused oncogenic transformation. High expression levels, however, are not the only pathway along which proto-oncogenes are converted to oncogenes.

Mutations in Oncogenes

Weinberg and his colleagues [8] at Cambridge, Mass., addressed the question as to whether cellular homologues of the viral oncogenes have a function in spontaneous, non-virally induced cancer. They transferred DNA extracted from animal carcinogen-induced cancers, and from human tumors, to cultured animal cells, and established that DNA from certain tumors indeed was able to transform the target cells, which as a result became oncogenic in nude mice. In one human tumor (bladder carcinoma) the transforming activity was found to reside in a gene that was virtually identical to the cellular c-Ha-ras gene (see Table 1). In contrast, the c-Ha-ras gene from normal human cells does not have transforming properties. The difference between the transforming Ha-ras and the wild type is caused by a single point mutation (G → T), as a result of which the 12th amino acid of the oncogenic ras polypeptide is valine instead of glycine. The work of many other groups subsequently indicated that some 15–20% of human and animal tumor DNAs had transforming activity in cultured NIH-3T3 mouse cells, and that in the majority of cases a ras gene was involved. The point mutations activating the ras gene affect only a few codons, i.e. nos. 12, 13 and 61 (viral ras genes in addition contain a mutation in codon 59). The transforming capacity of the various ras mutations is different and occasionally may be so weak that it is not detected in the transformation assay. This in part may explain why 80% of the tumor DNAs do not transform NIH-3T3 cells. Another explanation might be that some tumors carry mutations in other non-ras proto-oncogenes, which do not cause transformation of the NIH 3T3 target cells.

Activation of More Than One Proto-Oncogene May be Required to Generate a Cancer Cell

In recent years evidence has accumulated suggesting that for a normal cell to become cancerous, it may have to undergo more than one oncogene-activating step.

1. The human promyelocytic leukemia cell line HL60 carries a 20–30-fold amplified c-myc gene (being the cellular homologue of the v-myc gene of avian leukemia virus MC29). Due to the amplification, the myc RNA and protein concentrations are increased, suggesting a link between myc and the malignant state. Upon transfer of HL60 DNA to cultured mouse cells, transformed foci were observed but surprisingly, were found to be transformed not by human c-myc but by c-N-ras which was altered in its 61st codon. However, myc probably also contributes to the transformed state of HL60 cells, since treatment of these cells with the vitamin D3 derivative $1.25(OH)_2D3$ or with retinoic acid led to a drastic decrease in myc expression and concomitant-

ly, to differentiation into either granulocytes or monocytes [4]. These reversible effects demonstrate that the elevated myc expression at least partly is responsible for the oncogenic character of the HL60 cells.

2. In Burkitt's lymphoma, both c-myc and c-N-ras have been found to be implicated. The cells of this human B-lymphoid malignancy are characterized by specific chromosome (chr.) translocations, usually of a segment of the long arm of chr. 8 to the long arm of chr. 14, and vice versa. The translocation site in chr. 8 is in the close vicinity of the normal location of the c-myc gene, while the translocation point in chr. 14 invariably lies in the region coding for immunoglobulin heavy chains (Ig^H). Due to the translocation, the c-myc gene alights in the Ig^H domain (which in these B-lymphoid cells is transcriptionally highly active) and, as a result, is expressed continuously (constitutively), and possibly even at an elevated level. In normal circumstances, the myc gene is only active during the differentiation of pre-B-cells to Ig-producing cells, and is shut off a few cell divisions before terminal differentiation to a plasma cell in mature B-cells. The N-ras gene was found to be activated when Burkitt cell DNA was used to transform NIH 3T3 mouse cells. Consequently, two oncogenes appear to be involved in the generation of the Burkitt tumor.

3. The transformation assay discussed in the previous paragraphs used a *cell line* (NIH 3T3 mouse cells) as target cells. As stated, this cell line could be stably transformed by the activated ras gene. In contrast, *primary* cultures of rodent cells (e.g. baby rat kidney or rat embryo) do not show that response. The 3T3 cells distinguish themselves from primary ones in having acquired an unlimited lifespan, i.e. in being "immortal". Primary cells also can obtain that status, but this requires a transforming event, either spontaneous or induced by a virus or carcinogen. Transfection of an activated (mutated) ras gene, in combination with an activated myc gene (e.g. a myc gene under control of a retroviral LTR promoter) into *primary* rat embryo cells renders them oncogenically transformed. Presumably, the activated myc gene induces only the first stage of this process, i.e. immortalization, but it takes the combined action of the two genes to obtain tumor cells.

It is quite possible that the development of tumor cells to stages of higher malignancy, or to the metastasizing level, requires the action(s) of a third or fourth oncogene.

The Mode of Activation of Oncogenes

In the preceding paragraphs we have discussed three ways in which proto-oncogenes can be activated: mutation, translocation and amplification. Mutations, as the point mutations in ras genes, are probably introduced during semi-conservative replication, or caused by errors during repair of DNA damage. How the translocation or amplifications responsible for myc activation are caused, e.g. by the direct action of carcinogenic agents, is at present obscure. The order of the consecutive activation events is hardly more clear. Some observations would indicate that ras activation occurs at late stages of oncogenesis. Albino et al. [1] report that a human melanoma did not carry a detectable ras oncogene in the primary tumor, whereas some of its metastases did harbour an activated ras gene. On the other hand, Balmain et al. [2] detected ras activation in a benign papilloma, which suggests that it may also occur at early stages of oncogenesis.

Tumor Specificity of Oncogene Activation

No clearcut tumor specificity can be assigned to activated oncogenes. There is a tendency for activated N-myc (related to c-myc) to be found in neuroblastoma and in certain types of lung carcinoma, and for the activation of N-ras in haematopoietic tumors. Barbacid and co-workers (Guerrero et al. [3]; Zarbl et al. [5]), recently established that some carcinogens induced specific tumors in rats and mice and that each type of tumor (induced by a particular agent) carried a particular activated ras gene. For example, the carcinogen NMU in mice induced lymphomas in which only N-ras is activated, and in rats mammary carcinomas harboring activated H-ras; DMN induced kidney carcinomas in rats in which K-ras was activated. How this specificity occurs is yet not known. Possibly, certain ras genes have an elevated activity in some organs during certain phases of development, and therefore in these tissues are more sensitive to the action of oncogenes. Another explanation might be that carcinogens tend to accumulate in certain organs or tissues, thus increasing the chance of mutations occurring in the ras genes that are active in those tissues.

Functions of Proto-Oncogenes

The evidence for cellular proto-oncogenes is a recent discovery, and their function in most cases is still a matter of conjecture. However, new facts have come to light which demonstrate that at least some proto-oncogenes have a role in cellular growth and differentiation. For example, comparative sequence studies revealed that the proto-oncogene c-sis codes for one of the polypeptide chains of platelet-derived growth factor (PDGF), a growth hormone required for the proliferation of fibroblastic cells. In a similar way, v-erb-B was found to derive from a cellular gene encoding the receptor for epidermal growth factor (EGF), a receptor present on a large variety of cells. Stimulation of quiescent cells with PDGF induces a rapid and transient increase in the expression of two proto-oncogenes, c-fos (after ca. 20 min) and c-myc (after 2–4 h). In addition, there is evidence that src, ros and ras under normal conditions have a function in the transmission of signals caused by the binding of extracellular factors to their respective receptors on the cell membrane. As stated before, proto-oncogenes can be activated to cancer genes either by mutations of the oncogene

product itself (e.g. the ras genes) or by a change in the surroundings of the gene, causing it to escape the normal regulatory controls (e.g., myc in Burkitt cells). The activation by mutation could retain the ras gene products constantly, or at least longer than normal, in their active form so that they continue transmitting signals to the inner cell, even in the absence of an external stimulus. Another example of an oncogene which is activated by mutation is the v-erb-B gene. In this case, activation is caused by deletions leading to N-terminal and C-terminal translations of the erb-B product. Oncogene activation is sometimes simply caused by the turning-on of the expression of a gene, as in the case of the gene for PDGF (see also Table 1).

Hopefully, this brief survey will have shown that the discovery of oncogenes represents a breakthrough in cancer research enabling us to understand the molecular basis of cancer; a concerted effort will still be necessary before we can understand the mechanisms of carcinogenesis.

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