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Review

Tumor-suppressor genes: News about the interferon connection

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ABSTRACT **The interferons are a family of secreted, multifunctional proteins which are components of the defenses of vertebrates against viral, bacterial, and parasitic infections and certain tumors. They exert their various activities by inducing the synthesis of a large variety of proteins. There are direct and indirect indications that several of these proteins may have tumor-suppressor activities. The interferon-inducible proteins implicated include: (*i*) a double-stranded RNA-activatable protein kinase that can phosphorylate and thereby inactivate the eukaryotic peptide chain initiation factor eIF-2; (*ii*) the interferon regulatory factors IRF-1 and IRF-2, which can modulate the expression of the interferons and of some interferon-inducible proteins; and (*iii*) RNase L, a latent endoribonuclease which can be activated by (2′-5′)oligoadenylates, the products of a family of enzymes which are also interferon-inducible. It is noteworthy that some of the proteins encoded by tumor virus oncogenes (e.g., E1A from adenovirus, EBNA-2 from Epstein–Barr virus, and terminal protein from hepatitis B virus) impair the induction of at least some proteins by interferons.**

The tumor (or growth)-suppressor genes (e.g., *Rb*, p53, *APC*, *NF1*, *WT1*, *DCC*) encode proteins whose lack or inactivity may contribute to uncontrolled cell proliferation and tumor formation (for a review see ref. 1). Recent reports have added to the list of tumor-suppressor genes several genes whose expression is controlled by interferons (2–5).

Discovered in 1957 as biological agents interfering with virus replication (hence the designation), the interferons were shown later to be a family of multifunctional, secreted proteins (for reviews see refs. 6–9). In humans, 14 types of α-interferons and one type each of β- and γ-interferons have been identified. The interferons are components of the defenses of vertebrates against viral, bacterial, and parasitic infections and certain tumors. They modulate the functioning of the immune system in various ways and affect cell proliferation and differentiation. Treatment with interferons proved to be beneficial in various human afflictions of viral origin (e.g., chronic infection with hepatitis B or C viruses, juvenile laryngeal papillomatosis, condyloma acuminatum) and certain malignancies (e.g., hairy cell leukemia, Kaposi sarcoma) (for a review see ref. 10).

The interferons exert their multiple activities by binding to specific cell surface receptors and thereby triggering signals that result in the increased expression of many (over 30) genes (for a review see ref. 11). The α- and β-interferons on the one hand, and γ-interferon on the other hand, activate distinct, but overlapping, sets of genes. The genes activated encode proteins that mediate the various actions of the interferons.

Double-stranded RNA-Activatable Protein Kinase: Indications for Tumor-Suppressor Activity. One of the interferon-inducible proteins with an apparent tumor-suppressor activity is a double-stranded RNA-activatable protein kinase (recently designated as PKR) (for reviews see refs. 2 and 3). This cytoplasmic enzyme is also expressed constitutively at a low level in a large variety of cells. PKR is latent (as tested *in vitro*) unless activated by double-stranded RNA, single-stranded RNA with double-stranded segments, or some polyanions. The identity of the endogenous activator(s) is under investigation (12). Upon activation, PKR autophosphorylates on a serine residue in an apparently intermolecular reaction. The phosphorylated PKR can phosphorylate a serine residue of the α subunit of the eukaryotic peptide chain initiation factor eIF-2. The phosphorylated eIF-2 α subunit sequesters the guanine nucleotide exchange factor eIF-2B. This, in turn, prevents the recycling of eIF-2–GDP to eIF-2–GTP and, thereby, impairs protein synthesis.

The expression of the wild-type human PKR cDNA in transfected yeast slows down proliferation by phosphorylating eIF-2. Furthermore, the human PKR shares similar sequences with GCN2, a yeast protein kinase (13). Both enzymes are activated by RNA: PKR, as noted above, by double-stranded RNA and GCN2 (which stimulates the expression of amino acid biosynthetic genes under conditions of amino acid starvation) by uncharged aminoacyl-tRNA (14).

The transfection into murine (NIH 3T3) cells of wild-type human PKR cDNA resulted in partial resistance to infection by a virus (encephalomyocarditis virus), and the double-stranded RNA activating the PKR was most probably the replicating viral RNA (15). The transfected PKR cDNA was also shown to decrease nonviral protein synthesis in the cells (16).

The transfection into murine cells of a mutant human PKR cDNA encoding a protein lacking eIF-2 kinase activity [in consequence of the replacement of a crucial lysine residue (ref. 3) or the deletion of a crucial six-amino acid segment (ref. 2)] resulted in malignant transformation. When injected into nude mice, these cells produced large tumors within 1–4 weeks. In contrast, no tumor growth was observed during this time in mice injected with cells carrying only the endogenous PKR or cells transfected with wild-type PKR cDNA. Only much later, very few of the mice injected with the cells carrying the transfected wild-type PKR cDNA did form tumors. The tumor cells recovered were, however, shown to contain PKR devoid of activity. This suggests that naturally occurring mutations in the transfected PKR cDNA might have been responsible for the tumorigenesis (3).

These results served as the basis of a hypothesis stating that wild-type PKR is a tumor-suppressor gene product whose activity can be inhibited by the presence of catalytically inactive (dominant negative) PKR mutants (2, 3). It is not known whether the impairment is due (*i*) to the sequestering by the mutant PKR of phosphorylatable substrates involved in tumor suppression or of the agent (e.g., double-stranded RNA) activating PKR, (*ii*) to the formation of a hybrid (wild-type PKR–mutant PKR) protein complex which is inactive as a kinase, or (*iii*) to other causes. It may be relevant to the proposed role of PKR as a tumor suppressor that the oncogenic Ras protein was reported to induce an inhibitor of PKR activation (17).

The mechanism of the hypothetical antitumor action of wild-type PKR remains to be established. The most obvious possibility is the control of peptide chain initiation by phosphorylation of eIF-2. It is in line with this possibility that a loss of

Abbreviations: EBNA-2, Epstein–Barr virus-encoded nuclear antigen 2; eIF, eukaryotic initiation factor; IL, interleukin; IRF, interferon regulatory factor; PKR, double-stranded RNA-activatable protein kinase.

the control of peptide chain initiation—in consequence of the overexpression of another peptide chain initiation factor, eIF-4E—was found to give rise to tumorigenic cells (ref. 18; see also ref. 19). eIF-4E is a protein that binds to the 5′ cap structure of mammalian mRNA. An increase in the level of eIF-4E (which is part of a protein complex with RNA helicase activity) was found to increase preferentially the translatability of mRNAs with a strong secondary structure in their 5′-terminal region (20). The fact that numerous mRNAs that encode proteins enhancing cell proliferation (e.g., growth factors and protooncogenes) have such structures might account for the tumorigenicity of the overexpression of the eIF-4E factor.

In spite of this possible analogy, a mechanism (of the hypothetical antitumor activity of PKR) which is based on the control of eIF-2 phosphorylation still remains to be proven. This is the case especially since it was reported that the transfection of the mutant PKR cDNA into cells (which resulted in tumorigenicity) did not diminish the extent of eIF-2 phosphorylation (3).

A further conceivable mechanism might be based on the report (21) that, at least *in vitro*, PKR can activate the transcription factor NF-κB by phosphorylating, and thereby inactivating, IκB, its regulatory subunit (21). NF-κB and the related transcription factors are involved in the activation of a large number of genes (22). These genes might include the mediators of the tumor-suppressing activity of PKR.

It needs to be emphasized that the proposed tumor-suppressor activity of PKR is based at present primarily on experiments with mutant PKR. It will be important to establish the effect of knocking out the PKR gene on the growth characteristics of cells and organisms.

Interferon Regulatory Factor 1 (IRF-1) and Its Tumor-Suppressor Activity. IRF-1 is another protein which can be induced by interferons and some other cytokines [e.g., tumor necrosis factor α, interleukin 1 (IL-1), IL-6, and leukemia inhibitory factor (LIF)] and which has demonstrated tumor-suppressor activity (4, 23). IRF-1 is a transcriptional activator which has an antagonistic transcriptional repressor, IRF-2 (4). These two interferon-inducible agents are structurally related DNA-binding proteins that recognize the same oligodeoxynucleotide sequence in DNA. The sequence occurs in the promoters of the α-interferon and β-interferon genes and in those of many interferon-activatable genes. Consequently, the two proteins are regulators of both the induction and the actions of the interferons (24).

Both IRF-1 and IRF-2 mRNAs are expressed constitutively at a low level in various types of cells. The IRF-1 protein is much less stable than the IRF-2 protein (half-life of 30 min and 8 hr, respectively). Primarily, in consequence of this difference, the level of IRF-2 is higher than that of IRF-1. Exposure of cells to interferon induces IRF-1 first and IRF-2 only later. Thus, after exposure to interferon, there is a transient increase in the proportion of IRF-1 relative to IRF-2. This, in turn, contributes to a transient increase in the transcription of numerous interferon-activatable genes (4).

The level of IRF-2 mRNA is essentially unchanged in growth-arrested and in growing cells passing through the cell cycle. The level of IRF-1 mRNA is, however, high in serum-starved growth-arrested cells, and after serum stimulation it drops (to one-sixth of the earlier level) before starting to rise again.

When the IRF-2 gene was overexpressed in transfected murine (NIH 3T3) cells, these cells became transformed and tumorigenic when injected into nude mice. The transformed phenotype of such cells could be reversed to normal by overexpressing IRF-1 in them. Thus, it is the proportion of IRF-1 and IRF-2 which appears to exert growth control (4).

It was proposed that the inhibition of cell growth by interferons may be due, at least in part, to the transient induction of IRF-1 and that IRF-1 may function by activating sets of genes involved in the negative control of cell proliferation. IRF-2, whose overexpression results in tumorigenicity, may act by impairing the expression of such growth-controlling genes (4). The set of such genes might include the gene encoding PKR.

The disruption of the IRF-1 gene by homologous recombination in murine cells was reported recently (25). It will be of great interest to see the effect of this disruption on the growth characteristics of mice or at least murine embryos.

The tumor-suppressor activity of the IRF-1 gene is consistent with the fact that it has been localized at a chromosomal site that is frequently deleted in human leukemia (5). The gene maps to chromosome 5q31.1 (between the IL-5 and CDC25 genes). Its site is included in the smallest commonly deleted segment among deletions with different endpoints within the long arm of chromosome 5. Such deletions occur in 30% of patients with preleukemic myelodysplastic syndromes and in 50% of patients with acute myelogenous leukemia arising from myelodysplastic syndromes. Furthermore, the IRF-1 gene was the only gene consistently deleted at one or both alleles in samples from 13 patients with leukemia or myelodysplasia with aberrations of 5q31. In view of the finding that small changes in the ratio of IRF-1 to IRF-2 may perturb cell growth control, it was proposed that even loss of a single IRF-1 allele might have biological significance by providing growth advantage.

RNase L, a Candidate for Tumor-Suppressor Activity. The cDNA encoding RNase L, an interferon-inducible ribonuclease, was cloned recently (26). This enzyme is expressed constitutively at a low level in all cells tested. RNase L remains latent unless activated by 2′ → 5′-linked oligoadenylates. These, in turn, are synthesized by a family of interferon-inducible, (2′-5′)oligoadenylate synthetases which are latent unless activated by double-stranded RNA. Activated RNase L is an endoribonuclease which was shown to cleave various RNAs including rRNA and was implicated in the inhibition of encephalomyocarditis virus replication by interferon (for a review see ref. 9).

There are numerous indications for a role of RNase L in the control of cell growth and differentiation. Thus, for example, RNase L and (2′-5′)oligoadenylate synthetase levels are elevated in growth arrested cells and during cell differentiation. Furthermore, introduction of (2′-5′)oligoadenylates into cells (resulting in RNase L activation) impairs cell growth (7, 9, 26). There is a limited sequence similarity between RNase L and *E. coli* RNase E in a 200-amino acid segment. RNase E is required for efficient mRNA turnover and rRNA processing (27).

In view of the above considerations, there is a good chance that the RNase L gene might have tumor-suppressor activity. The availability of a cDNA clone should make possible definitive tests on the functions of this gene.

Impairment of Interferon Action by Tumor Viruses and Their Oncogenes. The significance of interferons and interferon-inducible proteins in tumor suppression might be reflected by the fact that some DNA tumor viruses impair interferon action (28, 29, 38). The antiinterferon activity is exerted in certain cases by the oncogenes of the tumor viruses. Thus, in adenovirus it is the E1A oncogene that inhibits gene activation by α-, β-, and γ-interferons, apparently by impairing the formation of an active transcription factor complex (ISGF-3) (29–31). E1A is the oncogene implicated in controlling the expression of a large variety of cellular and viral genes on the one hand and in the immortalization of cultured cells on the other hand.

Epstein–Barr virus is associated with benign and malignant B-cell hyperproliferation, nasopharingeal carcinoma, and Burkitt lymphoma. In the case of this virus, the same oncogene [encoding Epstein–Barr nuclear antigen 2 (EBNA-2)] that is involved in the immortalization of B cells (i.e., the induction and maintenance of B-cell proliferation) is also involved in making the proliferation of such

cells resistant to α-, β-, and γ-interferons (32–34). EBNA-2 encodes a protein which is located in the nucleus and functions as a transcription factor enhancing the expression of several viral and host genes. The EBNA-2 requirement for immortalization of B lymphocytes was demonstrated with immortalization-defective deletion mutants of Epstein–Barr virus (e.g., P3HR1) which lack the EBNA-2 gene. While the P3HR1 virus can undergo lytic replication, it cannot immortalize B lymphocytes. This defect in immortalization could be cured by complementation or recombination with a vector encoding EBNA-2 (32, 33).

The interference of the EBNA-2 gene with the interferon-induced antiproliferative response in human B-lymphoblastoid cell lines was uncovered by the finding that lymphoblastoid cells immortalized by complete Epstein–Barr virus were resistant to the antiproliferative effect of α-interferon, whereas lymphoblastoid lines carrying Epstein–Barr virus deletion mutants lacking the region encoding EBNA-2 were susceptible to this effect (34). Subsequently, it was demonstrated that the introduction of a vector carrying the EBNA-2 gene into human B-cell lines free of Epstein–Barr virus (or carrying Epstein–Barr virus genomes lacking the EBNA-2 region) resulted in conversion of these cells from sensitivity to the antiproliferative effect of α-interferon to resistance. It is intriguing that it was also reported that the introduction of the EBNA-2 gene into these lines did not impair the antiviral (specifically antivesicular stomatitis virus) activity of interferon (34).

The effect of EBNA-2 on the interferon inducibility of four genes was tested in two lymphoblastoid cell lines differing only by the presence of EBNA-2 in only one of the lines (35). The interferon-induced increase in the mRNA levels corresponding to these four genes was strongly reduced (in some cases abolished) in the cell line with EBNA-2, compared to the increase in the cell line without EBNA-2. So was the expression of a marker enzyme (chloramphenicol acetyltransferase) encoded by a transfected construct driven by various interferon-responsive promoters [interferon-stimulated response element (ISRE) and γ-interferon activation site (GAS)]. Unexpectedly, the activation of the ISGF-3 transcription factor complex was unaffected by the presence of the EBNA-2 gene (35). This transcription factor complex, which mediates the interferon-induced transcription of numerous genes, consists of three ISGF-3 α proteins and one ISGF-3 γ protein. The activation of the ISGF-3 transcription factor complex was tested in an electrophoretic mobility-shift assay involving the use of the ISRE to which the activated ISGF-3 complex binds.

These results seem to indicate that the impairment of interferon-inducible gene activation by EBNA-2 occurs downstream from the assembly of the ISGF-3 transcription factor complex. This is clearly a different mechanism from those used by adenovirus E1A protein (29–31) and hepatitis B virus terminal protein (36) to impair the activation of genes by interferons. Both of these viral proteins impair the assembly of the ISGF-3 transcription factor complex.

A commentary from 1990 entitled "Interferons, a New Class of Tumor Suppressor Genes?" carries a question mark in its title (37). Were we to consider the interferon genes together with the genes whose expression they control, then the recent findings appear to warrant that now the question mark be replaced by a period.

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