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# REVIEW ARTICLE

# ONCOGENES AND ONCO-SUPPRESSOR GENES: THEIR INVOLVEMENT IN CANCER

DEMETRIOS A. SPANDIDOS*† AND MARGARET L. M. ANDERSON‡

**The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, Scotland, U.K.; †Biological Research Center, National Hellenic Research Foundation, 48 Vas. Constantinou Ave., Athens 11635, Greece; ‡Department of Biochemistry, University of Glasgow, Glasgow, Scotland, U.K.*

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## SUMMARY

We review the involvement of two groups of genes, oncogenes and onco-suppressor genes, in malignant transformation. Approximately 40 oncogenes have been described mainly through studies on retroviruses and by *in vitro* functional analyses such as transfection of transforming genes into 'normal' cells. Because they are more difficult to identify, only a handful of onco-suppressor genes have been described so far, but potentially they could number as many as oncogenes. Where these genes have been isolated and sequenced, they have been shown to be highly conserved among species, suggesting that these genes play an essential role in the normal cell. Although some properties of oncogenes have been identified, we do not know in detail the role these genes play in normal cells or how genetic damage contributes to malignancy. The effect of oncogene expression on a cell depends both on the cell type and on the oncogene, and in some circumstances oncogenes act as onco-suppressor genes and vice versa. The elucidation of the mechanism of action of oncogenes and onco-suppressor genes will not only increase our understanding of these important genes but might also provide the framework for a biological approach to the treatment of cancer.

KEY WORDS—Oncogenes, onco-suppressor genes, carcinogenesis.

## INTRODUCTION

The evolution of a normal cell into a cancer cell is a complex multi-stage process that leads eventually to the emergence of a clone of cells which no longer has the same growth control restraints that affect normal cells. Analyses of the involvement of genetic damage in tumorigenesis have implicated three broad classes of genes. The first class are the oncogenes, a term originally coined by Huebner and Todaro.[1] These genes are derived by alterations of normal genes, proto-oncogenes, such that they become 'activated'.

A second class of gene confers predisposition to cancer when mutated. These genes are mainly involved in DNA repair[2] and they are not the subject of this review.

The third class, for which we propose the term 'onco-suppressor gene', is a very diverse group of genes which share the property that their expression inhibits the cancer phenotype. For some, it has been shown that inactivation or deletion of both alleles is a step in tumour formation.[3,4] Various terms have already been used to describe different members of this broad class, e.g., anti-oncogene,[3] tumour-suppressor gene,[5] emerogene.[6] It is to avoid confusion with the term suppressor as used in prokaryotic genetics and to be in accord with the oncogene nomenclature that we suggest the term onco-suppressor for this entire group of genes.

Available evidence shows that oncogenes and onco-suppressor genes are highly conserved in different species, consistent with their playing an important role in cellular physiology. In this paper

Addressee for correspondence: Professor D. A. Spandidos, Biological Research Center, National Hellenic Research Foundation, 48 Vas. Constantinou Ave., Athens 11635, Greece.

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we review the evidence that oncogenes and oncosuppressor genes are involved in the regulation of normal cell growth and differentiation and that mutations in these genes lead to defects in these processes.

## ONCOGENES

### *Identification*

Several approaches have led to the identification of genes whose expression contributes to the cancer phenotype of cells. Much of our early knowledge of oncogenes came from studies of rapidly transforming retroviruses. These viruses transform susceptible cells with the same kinetics that they infect them and are consequently among the most potent carcinogens known. At least 20 viral genes that cause transformation have been isolated and characterized and these include the *ras, myc* and *erbB* oncogenes (for a review see ref. 7).

Hybridization studies using these viral oncogenes as probes established that they have close nucleotide sequence homology to cellular sequences (for a review see ref. 8) that we now call proto-oncogenes.

Cellular oncogenes were also identified by direct experimental search for genes which could transmit the malignant phenotype from tumour to normal cells. This approach was originally introduced by Spandidos and Siminovitch.[9,10] Subsequently, 'normal' NIH3T3 fibroblasts were transfected with DNA from tumour-derived cell clones or from fresh tumour tissue (for a review see ref. 11). Transformation was monitored by screening for markers such as focus formation, anchorage independence, and tumorigenicity. The transforming agents were cloned and sequenced and in some cases were found to be homologous to oncogenes already identified in retroviruses, e.g., H-*ras* and K-*ras*. However, other oncogenes had not been previously identified, e.g., *neu* and N-*ras*.[11]

Studies of chromosomal aberrations in tumour cells have also implicated oncogenes in certain types of cancer. Lymphomas and leukaemias have been studied most extensively because of the ease of obtaining dispersed tumour cells. Consistent specific translocations are observed in these cancers. By molecular cloning, *myc* was invariably found at the translocation breakpoint in Burkitt's lymphoma and *abl* at the breakpoint of chronic myelogenous leukaemia (CML) (for a review see ref. 12).

The overlap between oncogenes identified by retroviral transformation, gene transfer assays, and chromosomal translocations suggests that the number of cellular genes with oncogenic potential is rather limited.

### *Involvement in cancer*

If normal proto-oncogenes are converted into potentially tumorigenic oncogenes they are said to be activated. This can occur in a variety of ways.

*(i) Transduction*—Retroviruses contain RNA as their genetic material. During infection, these viruses synthesize a double-stranded DNA copy of the RNA that integrates into the cellular DNA. Occasionally, recombination between viral and host DNA leads to incorporation of host sequences within the viral DNA. On subsequent production of virus, the RNA genome contains these cellular sequences usually at the expense of a viral sequence. Comparison of viral and cellular sequences shows that the transduced sequence is damaged compared with its normal cellular counterpart, often containing point mutations, deletions, and substitutions (for a review see ref. 13).

*(ii) Insertional mutagenesis*—Slowly transforming retroviruses can integrate into cellular DNA at many places, though probably not at random. At very low frequency, they may integrate next to a cellular proto-oncogene and cause its transcriptional activation through regulatory sequences in the long terminal repeat (LTR) of the viral DNA (for a review see ref. 13).

*(iii) Chromosome translocation*—Translocations are a common feature of human haematological malignancies and there is a large body of evidence suggesting that proto-oncogenes situated at the breakpoint are activated as a consequence of translocation.[12] Two different mechanisms of activation have been observed. The first is exemplified by chronic myelogenous leukaemia (CML).

In over 90 per cent of patients with CML, malignant cells carry a translocation t(9:22). The position of the breakpoint on chromosome 9 is variable, but occurs in a large region within the c-*abl* proto-oncogene.[14] On chromosome 22, the breakpoints cluster within two introns of a gene formerly called *bcr* (breakpoint cluster region), but now known as *phl*.[15] These translocations create a hybrid gene which is transcribed and translated to give a

chimeric protein with the amino terminus of the *phl* protein and the carboxy terminus of the *abl* protein.

This hybrid protein can autophosphorylate on tyrosine residues *in vitro*, a property not possessed by either the *phl*- or *abl*-encoded proteins alone.[15] Thus, the substrate specificity of the c-*abl* protein seems to be altered by replacement of its N-terminal sequences by *phl* sequences. It is possible that the acquisition of this altered property by the *phl–abl* protein may be pathogenic.

A completely different means of oncogene activation occurs in Burkitt's lymphoma. In virtually all patients with this disease, translocations juxtapose the *myc* oncogene on chromosome 8 to one of the immunoglobulin loci—either IgH on chromosome 14, or Igk on chromosome 2, or Ig$\lambda$ on chromosome 22. The position of the breakpoint with respect to the *myc* gene is variable, occurring 5′ to exon 1, between exons 1 and 2, or 3′ to coding region depending on the other chromosome involved in the translocation.[12] Similarly, the position of the breakpoint on the chromosome containing the immunoglobulin gene is variable.[12]

The constant feature displayed by all Burkitt's lymphomas is that the *myc* gene on the derivative chromosome is expressed constitutively whereas that on the normal chromosome 8 is transcriptionally silent. It is suspected, therefore, that oncogene activation in this disease involves expressing *myc* in cells which would not otherwise express it. Presumably some feature of its new chromosomal environment overrides the normal regulatory control of the *myc* gene.

*(iv) Mutation*—The human H-*ras*1 gene isolated from a bladder carcinoma cell line was the first cellular oncogene where activation was shown to occur by a point mutation (for a review see ref. 16). Subsequent studies have shown that in both cell lines and fresh cancer tissue, point mutations are common in *ras* gene coding sequences. Functional analysis of *ras* genes *in vitro* have indicated that only a limited range of mutations results in activation of these genes.[16] Using oligonucleotide probes, it has been found that as many as 40 per cent of human primary colon tumours exhibit structural *ras* gene mutations.[17,18] Similarly, activating *ras* gene mutations have been found in primary human breast cancer[19] and in human myelodysplasias.[20] Using monoclonal antibodies and high-resolution two-dimensional gel electrophoresis, mutant *ras* proteins have also been detected in human acute leukaemia.[21]

*(v) Amplification*—Proto-oncogenes are amplified in a variety of human tumours.[22] Two patterns have been observed: as an occasional feature of some tumours and as a common occurrence in particular tumours. In breast cancer, c-*myc* and *erb*B[23] or *neu*[24] oncogene amplifications have been consistently observed with the advanced but not earlier tumour stages and are associated with poor prognosis. Amplification of N-*myc* is a common feature of late stage neuroblastomas[25] and related tumours,[26] where up to 300 copies of N-*myc* gene per cell are present. N-*myc* amplification in these tumours is associated with a more aggressive stage of disease as characterized by a tendency to metastasize and shorter survival.

## ONCO-SUPPRESSOR GENES

A lack of selection systems for onco-suppressor genes has made it difficult to isolate and characterize these genes. At present, fewer onco-suppressor genes have been characterized than oncogenes, probably not because there are fewer oncosuppressor genes in nature, but because of the difficulty in detecting them. This is reflected in the fact that most onco-suppressor genes have been identified indirectly.

### *Heritable predisposition to cancer*

The number of genes whose inactivation through mutations predisposes to cancer is not known. However, it has been noted that approximately 9 per cent of the known single gene traits predispose to neoplasia.[27] It has also been estimated that approximately 10 per cent of human cancers can be attributed to an inherited genetic component together with a somatic mutation in the other allele.[28] The Mendelian inheritance of predisposition to cancer can be divided into two groups. The first group is composed of recessively inherited disorders such as ataxia telangiectasia, Bloom's syndrome, and Fanconi's anaemia. Patients with these disorders have defects in DNA repair and this leads to a higher than normal incidence of particular cancers (for a review see ref. 3). Attempts to identify the defects by complementation analysis have met with singularly little success. The second group is composed of dominantly inherited disorders, and is composed of the onco-suppressor genes.

The detection of some onco-suppressor genes was made possible by a combination of familial studies, cytogenetics, and molecular genetics involving restriction–fragment length polymorphism

Table I—Chromosomal assignment of human onco-suppressor genes determined by familial, cytogenetics, and RFLP studies

| Disease (gene) | Chromosome |
|---|---|
| Retinoblastoma (RB) | 13 |
| Wilms' tumour (WT) | 11 |
| Familial adenomatous polyposis (FAP) | 6 |
| Bilateral acoustic neurofibromatosis (BANF) | 22 |
| Multiple endocrine neoplasia type 1 (MEN-1) | 11 |
| Multiple endocrine neoplasia type 2 (MEN-2) | 1 |
| Multiple endocrine neoplasia type 2A (MEN-2A) | 10 |

(RFLP) analyses. Using this approach, the gene predisposing to retinoblastoma (RB) was identified.[29] This gene, encoding a messenger RNA (mRNA) of 4·6 kilobases (kb), located near the esterase D gene was cloned on the basis of chromosomal location, homozygous deletion, and tumour-specific alterations in expression in Rb patients.[30–32] The Rb product is a phosphorylated protein which binds to DNA, but its function is not known.

Onco-suppressor genes predisposing to Wilms' tumour (WT),[33] familial adenomatous polyposis (FAP),[34] bilateral acoustic neurofibromatosis (BANF),[35] multiple endocrine neoplasia type 1 (MEN-1),[36] multiple endocrine neoplasia type 2 (MEN-2),[37] and multiple endocrine neoplasia type 2A[38] have been assigned to particular chromosomes (see Table I), but the genes have not yet been isolated.

*Suppression of the transformed phenotype in cell hybrids*

It is now well established that when malignant cells are fused with diploid fibroblasts of the same species, the resulting hybrid cells are non-malignant as long as they retain certain specific chromosomes donated by the normal parent (for reviews see refs. 5 and 39). When these particular chromosomes are lost from the hybrid, through chromosome instability, the malignant phenotype reappears and the segregant cell is again able to give rise to tumours *in vivo*.

*Suppression of cancer cell growth and transformation phenotype by contact with normal cells*

It is known from the pioneering work of Stoker[40] that the growth and morphology of cells transformed by polyoma virus are influenced by contact with normal cells. Thus, when transformed and normal fibroblast cells were co-cultured, the growth of transformed cells was inhibited.[40] More recent work using cells transformed with the cloned human T24 H-*ras* oncogene and a drug selection system has shown that these results are not restricted to viral oncogenes.[41]

*Tumour inhibitory factors*

Certain proteins cause suppression of tumour cell growth and inhibit anchorage independence in *in vitro* assays. When injected *in vivo*, they also reduce tumorigenicity, invasion, and metastasis of cancer cells (for a review see ref. 6).

Transforming growth factor-$\beta$ ($\beta$-TGF),[42] interferons,[43] interleukin-2,[44] and tumour necrosis factor (TNF)[45] show these tumour inhibitory properties. The genes have been cloned and characterized and the biological properties of their protein products are under intensive investigation.

Several human tumour cells produce tumour cell inhibitory factors (TIFs). While these factors inhibit the growth of a variety of human tumour cells, they also stimulate the growth of normal cells. TIFs also inhibit anchorage independence, a property correlated with tumorigenicity. The effects of TIFs are reversible when the affected cells are no longer exposed to the factor.

## THE FUNCTION OF ONCOGENE AND ONCO-SUPPRESSOR GENE PRODUCTS

Although we know some properties of proto-oncogene products, we know very little about the function of these proteins in normal cells and even less about how activation of proto-oncogenes

influences the cancer phenotype. The high degree of conservation of proto-oncogenes and onco-suppressor genes among eukaryotes suggests that these genes serve important functions.

The properties and cellular location of the gene products of oncogenes and onco-suppressor gene products suggest several ways in which they might function (see Fig. 1 and Table II).

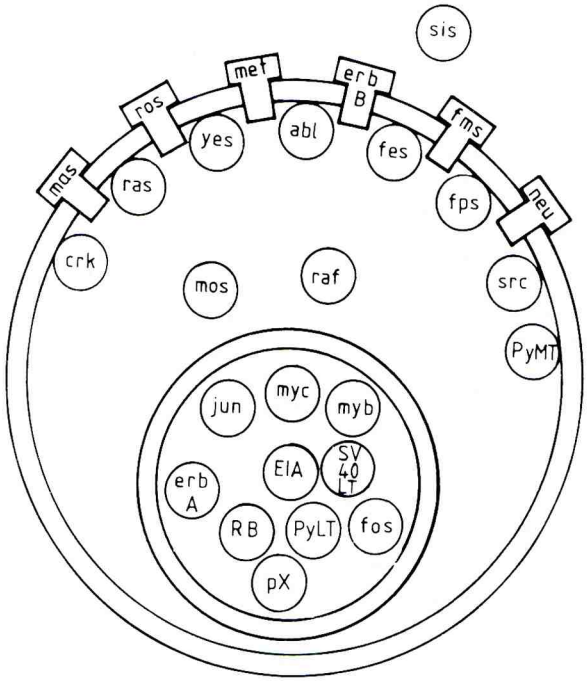


Fig. 1—Cellular location of oncogene and onco-suppressor gene products

(a) Some oncoproteins are structurally similar to secreted growth factors, e.g., the *sis* oncoprotein is partially homologous to the $\beta$-chain of platelet derived growth factor (PDGF).[46,47] Moreover, oncogene activation may lead to production of growth factors by the tumour cell itself, e.g., transforming growth factors (TGFs), and it has been suggested that autocrine mechanisms may play an important role in growth control of tumour cells.[48]

(b) Some oncoproteins are growth factor membrane receptors, e.g., the *erb*B oncoprotein is homologous to epidermal growth factor receptor,[49] and the *fms* oncogene product to the colony stimulating factor 1 receptor.[50]

(c) Oncoproteins such as the *ras, src, fps, abl, yes, fes, crk*, and the polyoma virus middle T antigen (PyMT) gene products are located in the internal part of the cytoplasmic membrane and are thought to be involved in mediating receptor signalling (for a review see ref. 51). These oncogene-encoded products may act immediately downstream from the receptors in a mitogenic signal-transducing pathway in the cytoplasm. There is substantial evidence to implicate the *ras* p21 protein in signal transduction.

(d) Proteins encoded by the *mos* and *raf* oncogenes are located in the cytoplasm.[51] They are protein kinases which phosphorylate proteins at serine and threonine residues, but their role in cell transformation is not known.

(e) Proteins encoded by the *myc, myb, fos, jun, erb*A, adenovirus E1A, the HTLV-I pX, the polyoma virus large T antigen (PyLT), the SV40 large T antigen (LT) and the retinoblastoma (RB) genes are located in the nucleus. Those encoded by *myc, myb, jun*, Rb, PyLT, and SV40 LT genes are phosphoproteins and bind to DNA. Their function in the cell is unknown, but their nuclear localization suggests that they might be involved in DNA replication, or transcription, or maintaining nuclear structure.[51]

The adenovirus E1A and the HTLV-I pX proteins are thought to act as transcriptional activators since they can stimulate transcription probably by interacting with components of the transcriptional complex.

The *jun* oncoprotein has been recently identified as a transcriptional trans-activator and is homologous to the mammalian transcriptional activator AP-1.[52]

At one time, oncogenes were thought to act at specific stages of carcinogenesis, e.g., *myc* as an early gene involved in immortalization of cells and *ras* as a late gene involved in tumorigenic transformation.[17] It is now known that oncogenes cannot be categorized in this way since there are many examples of a single oncogene, e.g., *ras* or *myc* being involved in several steps or stages of cell transformation. Thus, it has been found that both *ras* and *myc* are activated in benign or malignant tumours and cause immortalization of early passage cells, anchorage independence, and tumorigenicity.[53]

## DIFFERING EFFECTS OF ONCOGENES AND ONCO-SUPPRESSOR GENES

There are several examples of oncogenes and onco-suppressor genes eliciting different responses

Table II—Location and properties of oncogene and onco-suppressor gene protein product

| Gene | Product location | Biochemical properties |
|---|---|---|
| *Oncogene* | | |
| *Sis* | Secreted | Ligand for PDGF receptor |
| α-TGF | Secreted | Ligand for EGF receptor |
| *erb*B | Transmembrane | Protein kinase (tyr) |
| *fms* | Transmembrane | Protein kinase (tyr) |
| *neu* | Transmembrane | Protein kinase (tyr) |
| *ros* | Transmembrane | Protein kinase (tyr) |
| *met* | Transmembrane | Protein kinase (tyr) |
| *src* | Plasma membrane | Protein kinase (tyr) |
| *fps* | Plasma membrane | Protein kinase (tyr) |
| *fes* | Plasma membrane | Protein kinase (tyr) |
| *abl* | Plasma membrane | Protein kinase (tyr) |
| *yes* | Plasma membrane | Protein kinase (tyr) |
| *ras* | Plasma membrane | GTPase |
| PyMT | Plasma membrane | Complexed with c-src protein |
| *mos* | Cytoplasm | Protein kinase (ser/thr) |
| *raf* | Cytoplasm | Protein kinase (ser/thr) |
| *myc* | Nucleus | Binds DNA |
| *myb* | Nucleus | Binds DNA |
| *jun* | Nucleus | Binds DNA |
| PyLT | Nucleus | Binds DNA |
| SV40 LT | Nucleus | Binds DNA |
| *fos* | Nucleus | ? |
| pX | Nucleus | Transcriptional activator |
| E1A | Nucleus | Binds DNA |
| *erb*A | Nucleus | Analogue of steroid receptors |
| *Onco-suppressor gene* | | |
| RB-1 | Nucleus | Binds DNA |
| β-TGF | Secreted | Ligand for β-TGF receptor |

according to the lineage of the target cell. Some effects on cell growth and differentiation are shown in Table III.

Stimulation or inhibition of cell growth has been observed for several growth factor/receptor systems. Oncogenes such as *ras* and *fos* which are not growth factor/receptors also show this effect. Thus, these proteins act as oncogenes or onco-suppressor genes according to the target cell.

*Ras* is particularly interesting as it may play a role in cell differentiation as well as in proliferation (see Table III). Rat phaeochromocytoma cells proliferate indefinitely as chromaffin-like cells. Upon transfection of an activated *ras* gene[54] or microinjection of the transforming *ras* protein,[55] they differentiate into neuron-like cells, mimicking the effect produced by nerve growth factor.

## EPIGENETIC MECHANISMS OF CARCINOGENESIS

While there is abundant evidence that mutations are involved in several steps of carcinogenesis, it is likely that epigenetic events also contribute to the development of cancer cells. Epigenetic events are non-heritable and include modifications of DNA, RNA, and protein which can influence the cell phenotype (but not genotype). For example, genes which are being transcribed are usually hypomethylated (for a review see ref. 7).

Hypomethylated oncogenes have been detected in several tumours, e.g., *ras* genes in colorectal cancer.[56] Furthermore, *in vitro* methylation of an activated Ha-*ras* oncogene diminishes its transforming activity in 3T3 cells.[57] This clearly

Table III—Differing effects of oncogenes and onco-suppressor genes on cell proliferation and differentiation

| Gene | Target cell | |
|---|---|---|
| | Proliferation (induction or inhibition) | Differentiation (induction or inhibition) |
| *Oncogene* | | |
| *ras* | Fibroblast (induction) | Phaeochromocytoma (induction)<br>Erythroleukaemia (inhibition)<br>Fibroblast (inhibition) |
| *src* | Fibroblast (induction) | Phaeochromocytoma (inhibition) |
| *fos* | Fibroblast (induction) | Embryonal carcinoma (induction) |
| *Onco-suppressor gene* | | |
| β-TGF | Fibroblast (induction)<br>Epithelial (inhibition) | Fibroblast (inhibition)<br>Epithelial (induction) |
| TNF | Epithelial (inhibition) | Endothelial (induction) |

demonstrates that oncogene activity can be modulated by methylation.

Differentiation, which can be viewed as arising through a series of epigenetic changes, may alter the tumorigenic phenotype of cells.

Tumour promoters and progressors are compounds that affect carcinogenesis epigenetically. Some, such as phorbol esters, can induce unscheduled proliferation of certain cells, but differentiation of others.[58]

## APPLICATIONS OF ONCOGENE AND ONCO-SUPPRESSOR GENE RESEARCH TO MEDICINE

Although there are many causes of cancer, they seem to affect a relatively small number of cellular genes that we now call oncogenes and oncosuppressor genes. The application of molecular biology to cancer research has led to a vast increase in our understanding of the role of these genes in carcinogenesis. It is one of the aims of these studies to exploit the findings for the benefit of cancer patients.

Determining alterations in oncogenes and onco-suppressor genes in tumours may be useful for diagnosis, prognosis, and determining therapeutic regimes. Thus, molecular hybridization techniques involving DNA or RNA from tumours,[59] as well as immunohistochemical techniques,[60] may be used to assess quantitative and/or qualitative changes in the oncogenes and onco-suppressor genes and their expression. Monoclonal antibodies to oncogene products may also play a role in diagnosis since when linked to radionuclides they can be used to locate tumours.[61] Probing DNA from people at high risk for developing a malignancy such as familial polyposis coli could be performed[34] to determine if they carry the hereditary predisposition to the disease. Similarly, when probes become available, other recessive genes could be screened (see Table I).

Alterations in oncogenes and onco-suppressor genes may be used for prognosis. Thus, overexpression of *ras* in breast cancer[62] or *myc* in cervical[63] or breast[23] cancer or the EGF receptor (a homologue of the *erb*B oncogene) in breast cancer[23] has been shown to correlate with a poor prognosis.

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