Reprod Nutr Dev (1990) 30, 445-454

Review article

Oncogenes and anti-oncogenes in tumorigenesis

R Monier

Institut Gustave-Roussy, UA CNRS 1158, Laboratoire d'Oncologie moléculaire, 39, rue Camille Desmoulins, 94805 Villejuif cedex, France

(15th meeting of the INRA development group, Paris, 24–26 May 1989)

Summary — Recent advances have led to the identification of cellular genes which are involved in the initiation and progression of tumorigenesis. The proto-oncogenes, which normally participate in the regulation of cell proliferation and differentiation, can become oncogenes through alterations in the regulation of their expression and/or their coding sequences. Their contribution to the tumorigenic phenotype is dominant. The anti-oncogenes or tumor suppressor genes or recessive oncogenes are normally implicated in a negative regulation of cellular proliferation. The loss of their activity contributes to tumorigenesis in a recessive manner. Genetic events activating proto-oncogenes or inactivating anti-oncogenes accumulate in the same cell during tumor progression and co-operate to determine the malignant invasive phenotype of advanced tumors.

oncogene / anti-oncogene

Résumé — Oncogènes et anti-oncogènes dans la tumorigenèse. *L'utilisation des méthodes de la génétique moléculaire a conduit à l'identification de deux classes de gènes cellulaires, qui interviennent dans l'initiation et la progression des tumeurs humaines. Les proto-oncogènes participent à l'oncogenèse après activation par un événement génétique qui altère leur expression et/ou la structure de leur produit. Leur participation au déterminisme du phénotype tumoral présente un caractère dominant. Les anti-oncogènes, dont le prototype est le gène Rb impliqué dans la transmission héréditaire du rétinoblastome codent probablement pour des produits qui interviennent dans un contrôle négatif de la prolifération cellulaire. C'est donc leur perte ou leur inactivation qui intervient dans le processus oncogène, de façon récessive. L'exemple d'une tumeur humaine fréquente, le cancer du colon, montre que les inactivations d'anti-oncogènes sont au moins aussi importantes pour conduire à la malignité que les activations de proto-oncogènes.*

oncogène / anti-oncogène

INTRODUCTION

Recent progress, following the introduction of molecular genetics methods, has permitted the identification of some of the genes which might be involved in the initiation and progression of tumorigenesis. Some of these genes are cellular genes, the proto-oncogenes, which normally function in the regulation of cellular proliferation and differentiation. Through alterations in the regulation of their expression and/or in their coding sequences, these proto-oncogenes are activated into oncogenes, whose persistent expression contributes to the tumorigenic phenotype in a dominant manner.

The study of some cancers, which can occur in hereditary forms, has led to the identification of another class of genes,

whose alterations appear to be equally important in tumorigenesis. These so-called anti-oncogenes or tumor suppressor genes or recessive oncogenes are probably implicated in a negative regulation of cellular proliferation. Their normal activity therefore tends to limit cell growth and it is the loss of this activity which indeed contributes to tumorigenesis. The genetic events which affect them are recessive and both alleles of the gene in diploid cells need to be lost or otherwise rendered inactive, in order to contibute to the tumorigenic phenotype.

THE PROTO-ONCOGENES

More than 50 genes have been identified in animals and in human tumors as proto-oncogenes and there is no reason to believe that this list is closed. Many of these genes are recognized as proto-oncogenes because of their aptitude to transform cells in culture or to induce tumors in animal models or because they are frequently rearranged or amplified in human tumors, although their biochemical properties and biological functions remain unknown. Nevertheless, about half of the proto-oncogenes identified at the present time are known to code either for growth factors or for elements of the cellular machinery which enable cells to recognize growth factors and to translate the information communicated by growth factors into cellular responses (table I).

Genes coding for membrane receptors are well represented in the proto-oncogene class. All of them code for integral membrane proteins, with an extracellular receptor domain and an intracellular domain possessing a tyrosine protein kinase activity. The ligands for only 2 of these proto-oncogenes are known: the c-*erb*-B1 gene codes for the receptor of EGF and the c-*fms* gene codes for the receptor of M-CSF (CSF-1). All the other proto-oncogenes of this class are included in the receptor gene category, because of sequence similarities with known receptor genes. The lack of knowledge about possible ligands is partic-

Table I. Proto-oncogene products.

Proto-oncogene	*Product*
int-2, *hst*, *fgf*-5, *sis*	Growth factor
erb-B1, *erb*-B2, *fms*, *met*, *kit*, *trk*, *sea*, *ros*	Growth factor receptor with tyrosine-protein kinase activity
mas	Angiotensin receptor
src, *yes*, *fes*, *fps*, *fgr*, *abl*, *lck*	Membrane-bound tyrosine protein kinase
Ha-*ras*, Ki-*ras*, N-*ras*	Membrane-bound GTPase
mos, *raf*	Cytoplasmic serine-threonine-protein kinase
fos, *jun*, *myb*	Nuclear transcription factors
erb-A	Thyroid hormone receptor
myc	Nuclear phosphoprotein

ularly frustrating in the case of the c-*erb*-B2 gene, because of its implication in human breast tumorigenesis and of its importance as a marker with prognostic value (Slamon *et al,* 1987; Guérin *et al,* 1988; King *et al,* 1989; Tsuda *et al,* 1989; Wright *et al,* 1989).

Some proto-oncogenes, such as c-*fos*, c-*jun* or c-*myb* code for nuclear phosphoproteins, whose production and/or activity are modulated in response to the binding of growth factors to their membrane receptors.

A particularly interesting case is represented by the c-*erb*-A proto-oncogene, which codes for 1 form of the thyroid hormone receptor. Recent data (Damm *et al,* 1989) have shown that the activated oncogene, v-*erb*-A, transduced by the avian erythroblastosis virus, contributes to erythroblast transformation by blocking thyroid-hormone-induced differentiation. The v-*erb*-A product has kept the DNA binding activity of the normal thyroid-hormone receptor but has lost its ability to bind the thyroid hormone. As a consequence, v-*erb*-A functions as a constitutive repressor of triiodothyronine responsive genes. It represents therefore the first example of dominant negative oncogenes, predicted on theoretical grounds by Herskowitz (1987).

The precise function of the c-*myc* gene, whose activity is associated with cellular proliferation and which has so often been implicated in human tumors, still constitutes an enigma: it is not clear as yet as to whether its product directly participates in DNA replication (Iguchi-Ariga *et al,* 1987) or in the regulation of gene expression (Kingston *et al,* 1984) at the transcriptional (Onclerq *et al,* 1988) or post-transcriptional level (Prendergast and Cole, 1989).

Little is known about the precise pathways through which the signals transduced through the plasma membrane by membrane receptors are transmitted to the nucleus to activate gene expression and DNA replication. Protein kinases located in the cytoplasm are probably involved and one good candidate is the product of the c-*raf* proto-oncogene, which encodes such a serine-threonine protein kinase. Recent data (Morrison *et al,* 1988; Wasylyk *et al,* 1989) show that its kinase activity is increased in response to growth factor stimulation and that the expression of the *raf* oncogene activates transcription from promoter elements recognized by the products of the nuclear proto-oncogenes c-*jun*/c-*fos*.

Several proto-oncogenes code for proteins which bind to the cytoplasmic side of the plasma membrane, after post-translational addition to their peptide structure of a hydrophobic chain (Buss and Sefton, 1985; Hancock *et al,* 1989). Some of them (*eg* c-*src*) code for tyrosine-protein kinases, while the *ras* genes code for proteins of 21 kDa with sequence similarities to the α-subunit of G proteins which bind and hydrolyze GTP. The function of these proto-oncogenes remains unclear, although their implication in the process of signal transduction is probable. It has been suggested that tyrosine-protein kinases of the *src* family are partners of cytokine membrane receptors which have no tyrosine-protein kinase domain of their own. Recently, evidence supporting this hypothesis has been provided for c-*lck*, which is expressed in T-lymphocytes and whose kinase activity is increased in response to T-cell activating signals (Veillette *et al,* 1989).

THE ANTI-ONCOGENES

The existence of anti-oncogenes or tumor suppressor genes was postulated on the basis of 2 types of observations :

— it has frequently been observed that hybrid cells, resulting from the fusion of a tumor cell with a normal cell, are not able to grow as tumors when injected into immuno-compromised animals (Stanbridge, 1987). The tumorigenic phenotype in these experiments is therefore recessive and the normal cell is supposed to express genes, which suppress the tumorigenic phenotype.

— Evidence has been accumulating in recent years to the effect that the proliferation of normal cells is regulated by cytokines, some of which stimulate cell division while others exert a negative effect on cell growth (Sporn and Roberts, 1988). This dual type of regulation leads to the prediction that deregulated proliferation, as observed in transformed and tumor cells, could be the result either of an excess of proliferative signals or of the loss of negative regulation.

THE Rb GENE

The identification of the first anti-oncogene actually stemmed from the study of one particular cancer, retinoblastoma, which occurs either in hereditary or sporadic form in young children.

An epidemiological study of retinoblastoma enabled Knudson (1971) to propose that the appearance of the tumor requires 2 genetic events occurring successively in the same retina precursor cell. In hereditary cases, 1 of these 2 genetic changes is already present in the germ line and is the cause of the hereditary transmission of retinoblastoma. The second genetic event occurs in a somatic cell and, when it has occurred in a retina precursor cell, a retinoblastoma is produced. Further cytogenetic and gene linkage studies identified a locus on chromosome 13 p 14 as linked to the hereditary predisposition to retinoblastoma. The cloning of the retinoblastoma (Rb) gene (Friend *et al,* 1986; Lee *et al,* 1987) established that the 2 genetic events postulated by Knudson actually affect the same locus on each of the 2 chromosomes 13 in diploid cells and amount to the complete loss or inactivation of the 2 Rb alleles.

The *Rb* gene covers about 200 kb and is comprised of 27 exons (Hong *et al,* 1989; T'Ang *et al,* 1989). Its promoter region has no recognizable TATA or CAAT boxes, but possesses an Sp I transcription factor binding site, and a GC-rich domain, *ie* it has the characteristic features of house keeping gene promoters. The *Rb* gene is transcribed as a 4.6 kb mRNA, and its protein product is a 105 kDa phosphorylated polypeptide located in the nucleus. The Rb protein is subject to a cycle of phosphorylation-dephosphorylation which is coupled to the cell cycle (Ludlow *et al,* 1989).

Thus, in the retinoblastoma case, tumorigenesis is the result of the inactivation of 1 particular gene, the *Rb* gene, and not of the activation of proto-oncogenes. The *Rb* gene is at the moment the best example of a gene whose activity is antagonistic to tumor development, *ie* of an anti-oncogene or tumor suppressor gene.

The *Rb* gene, which is responsible for the hereditary predisposition to retinoblastoma, predisposes also to osteosarcomas and, possibly, melanomas as observed in survivors of hereditary retinoblastomas (Murphree and Benedict, 1984; Hansen *et al,* 1985). However, its inactivation may also take part in the progression of other tumors, since several recent observations have demonstrated that the *Rb* gene is not only inactivated in all retinoblastomas, but also in a fraction of small cell lung cancers (Harbour *et al,* 1988) and breast cancers (Lee *et al,* 1988; T'Ang *et al,* 1988).

Moreover, it has now been shown that the transforming proteins of several DNA tumor viruses (SV40 large T, Polyoma large T, Adenovirus 5 E1A, HPV-6 and HPV-16 E7) (De Caprio *et al,* 1988; Whyte *et al,* 1988; Dyson *et al,* 1989) bind to the unphosphorylated form of the Rb protein (Ludlow *et al,* 1989). Some mutants of SV40 T and adenovirus 5 E1A, which are inactive in transformation are also unable to bind the Rb protein. Therefore, it is likely that the formation of complexes between the viral transforming proteins and the Rb protein plays a role in transformation. It can be suggested that the viral protein act, at least in part, by preventing the *Rb* gene from playing its normal role in the regulation of cellular division.

THE p53 GENE

Another likely example of anti-oncogenes is the gene coding for the nuclear phosphoprotein p53. The *p53* gene was previously considered as a proto-oncogene, because it apparently co-operated with the Ha-*ras* oncogene in rodent cell transformation (Eliyahu *et al,* 1984; Parada *et al,* 1984). However, it was recently discovered that the particular p53 cDNA clone used in co-transformation experiments was a mutated form of the normal gene, which is itself devoid of co-operating activity (Hinds *et al,* 1989). Moreover, there are several examples of tumorigenic cell lines in which the *p53* gene is lost or otherwise inactivated (Wolf and Rotter, 1985; Masuda *et al,* 1987; Ben-David *et al,* 1988; Hicks and Mowat, 1988) and it has already been known for several years that p53 forms complexes with SV40 large T (Lane and Crawford, 1979) and adenovirus 5 E1B (Sarnow *et al,* 1982) in cells transformed by SV40 and adenovirus 5, respectively. It also forms complexes *in vitro* with the E6 protein of HPV16 (Howley, personal communication). For all these reasons, it is likely that the *p53* gene, which maps to human chromosome 17 p (Miller *et al,* 1986) is in fact an anti-oncogene and not a proto-oncogene (Finlay *et al,* 1989). If this is true, it shows that anti-oncogenes can be confused with proto-oncogenes, because some of their mutants are able to exert a dominant negative effect.

THE Krev-1/rap-1 GENE

Many attempts to find tumor suppressor genes by transfecting DNA from normal cells into transformed cell lines and looking for morphological revertants have been made. One of these attempts succeeded in identifying the murine *Krev*-1 gene, a gene which could code for a protein of 184 aminoacids with several similarities to Ha-*ras*-p21 and which can revert to "flat" morphology NIH 3T3 cells transformed by the Kirsten strain of murine sarcoma virus (Kitayama *et al,* 1989). The *Krev*-1 gene is identical to *rap*-1, a member of the *ras* gene family, previously cloned by Pizon *et al* (1988).

ANTI-ONCOGENES IN OTHER HEREDITARY TUMORS

Besides retinoblastoma, there are several other examples of tumors, the predisposition of which is transmitted from parents to children through the germ line. For some of them, linkage studies and DNA restriction fragment length polymorphism have permitted the identification of chromosome regions in which the predisposing genes are located (table II). At the moment, it is usually assumed that these genes should be anti-oncogenes as is the case for retinoblastoma.

Table II. Chromosome loci associated with inherited predispositions to cancers.

Tumor	*Chromosome loci*
Retinoblastoma/Osteosarcoma	13 q14
Wilms' tumor associated with:	
WAGR syndrome	11 p13
Beckwith-Wiedeman syndrome	11 p15
Multiple endocrine neoplasia, 2A	10 p11.2-q11.2
Neurofibromatosis Type 1	17 q12-q22
Neurofibromatosis Type 2	22 q11.1-q13.1
Polyposis coli	5 q21-q22
Von Hippel-Lindau syndrome	3 p25

Nevertheless, it should be pointed out that none of the hypothetical anti-oncogene has been identified and cloned as yet and there are indications that genetic mechanisms involved in hereditary tumors other than retinoblastoma might be more complicated. In the case of the Wilms' tumors of the kidney, 2 loci on chromosone 11, 1 at 11 p 13 and the other at 11 p 15 are linked to the genetic predisposition. The existence of a 3rd locus, which has not been located as yet, has recently been recognized (Grundy *et al,* 1988; Huff *et al,* 1988). It is equally possible that, in other hereditary tumors, the predisposing gene be of the proto-oncogene type, rather than of the anti-oncogene type.

ALLELE LOSSES IN HUMAN COMMON CANCERS

The use of restriction fragment length polymorphism has shown that, in many human tumors, large deletions of well-defined chromosome regions are frequently observed (table III). It is suspected that the chromosome regions where these deletions occur contain alleles of anti-oncogenes, which are thus lost in the tumor cells. In 2 cases, hypotheses can be formulated with regard to the identity of the postulated anti-oncogenes: allele losses on the long arm of chromosome 13 could affect the *Rb* gene while those occurring on the short arm of chromosome 17 could entail the loss of a p53 gene allele (Baker *et al,* 1989).

Colorectal cancer is a good example of a common human tumor, for which the respective contributions of oncogene activation and allele losses to tumor progression have been carefully studied (Vogelstein *et al,* 1988). Most colorectal carcinomas appear to arise from adenomas, which can themselves be classified in classes representing successive steps in the transition from the normal epithelium to the malignant tumor. Moreover, familial adenomatous polyposis is a hereditary condition which predisposes to the development of many colorectal adenomas in affected persons. The locus *(fap)* segregating with this disease has been mapped to the long arm of chromosome 5 (Herrera *et al,* 1986; Bodmer *et al,* 1987; Leppert *et al,* 1987). The determination in tumors at different levels of progression of the respective frequencies of allelic losses on chromosomes 5, 17 and 18 and of activation of the Ki-*ras*

Table III. Allelic losses in human tumors (detected by restriction fragment length polymorphism).

Tumor	*Chromosome sites*
Bladder	11 p
Breast	11 p, 13 q, 17 p
Colon	5 q, 17 p, 18 q
Gliomas (esp. astrocytomas)	17 p
Renal cell carcinoma	3 p14-p25
Lung	
small cell lung carcinomas	3 p14-p25, 13 q, 17 p
others	3 p, 11 pter-p15.5, 11 p13-q13, 13 q, 17 p
Parotid gland	3 p14-p25
Stomach	11 p
Thyroid (medullary)	1 p
Uterine cervix	3 p, 11 p

proto-oncogene has led Vogelstein *et al* (1988) to suggest that in patients without familial polyposis, the somatic loss of 1 allele at the *fap* locus could be the 1st event leading to an inefficient control of cellular proliferation. In many patients, the appearance of a fully malignant tumor would result from the accumulation in the same cell of the *fap* allele loss, a Ki-*ras* gene mutation, the allelic loss of chromosome 18 q sequences and the allelic loss of chromosome 17 p sequences occurring in that order.

Classical epidemiological studies of colorectal cancers have already suggested that the appearance of a malignant carcinoma requires the accumulation of 5–6 genetic events in the same cell (Cairns, 1978). Recent molecular genetic studies therefore confirm the multi-step nature of colorectal tumor progression and have permitted the identification of 4 different genetic alterations associated with these tumors. The fact that amongst these 4 alterations, 3 are likely to correspond to the loss of anti-oncogenes and 1 is a proto-oncogene mutation underlines the importance of anti-oncogene inactivation in 1 common cancer.

Another intriguing aspect of these data is that, even though *ras* mutations occur in about 50% of all tumors examined, it remains that, in the other 50%, no *ras* gene mutation was observed. The identification of the genetic event which can substitute for the *ras* gene mutation is evidently a high priority goal. Similarly, allelic losses on chromosomes 5, 18 and 17 do not occur in all tumors, nor do they always occur in the order described above for some of the tumors. It would be of evident clinical interest to distinguish between 2 possible hypotheses. The 1st hypothesis would suggest the existence of a panel of genetic alterations, some of which, in different combinations, would lead to the appearance of tumors with identical pathological presentations. The 2nd hypothesis states that different combinations of genetic alterations actually produce phenotypically different tumors that we are not able to recognize as pathological entities because of the relative inefficiency of our present techniques.

The second hypothesis is already supported by studies which attribute significant prognostic value to observations on the state and expression of some oncogenes in common human cancers.

REFERENCES

Baker SJ, Fearon ER, Nigro JM, Hamilton SR, Preisinger AC, Jessup JM, Van Tuinen P, Ledbetter DH, Barker DF, Nakamura Y, White R, Vogelstein B (1989) Chromosome 17 deletions and *p53* gene mutations in colorectal carcinomas. *Science* 244, 217-220

Ben-David Y, Prideaux VR, Chow V, Benchimol S, Bernstein A (1988) Inactivation of the *p53* oncogene by internal deletion of retroviral integration in erythroleukemic cell lines induced by Friend leukemia virus. *Oncogene* 3, 179-185

Bodmer WF, Bailey CJ, Bodmer J, Bussey HJR, Ellis A, Gorman P, Lucibello FC, Murday VA, Rider SH, Scambler P, Sheer D, Solomon E, Spurr NK (1987) Localization of the gene for familial adematous polyposis on chromosome 5. *Nature* 328, 614-616

Buss JE, Sefton BM (1985) Myristic acid a rare fatty acid is the lipid attached to the transforming protein of Rous sarcoma virus and its cellular homolog. *J Virol* 53, 7-12

Cairns J (1978) *In: Cancer: Science and Society.* Freeman, San Franciso, 37

Damm K, Thompson CC, Evans RM (1989) Protein encoded by v-*erb*-A functions as a thyroid-hormone receptor antagonist. *Nature* 339, 593-597

De Caprio JA, Ludlow JW, Fiage J, Shew JY, Huang CM, Lee WH, Marsilio E, Paucha E, Livingston DM (1988) SV40 large T antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. *Cell* 54, 275-283

Dyson N, Howley P, Münger K, Harlow E (1989) The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 243, 939-937

Eliyahu D, Raz A, Gruss P, Givol D, Oren M (1984) Participation of p53 cellular tumor antigen in transformation of normal embryonic cells. *Nature* 312, 646-649

Finlay CA, Hinds PW, Levine AJ (1989) The *p53* proto-oncogene can act as a suppressor of transformation. *Cell* 57, 1083-1093

Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, Dryja TP (1986) A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* 323, 643-646

Grundy P, Koufos A, Morgan K, Li FP, Meadows AT, Cavenee WK (1988) Familial predisposition to Wilms' tumour does not map to the short arm of chromosome 11. *Nature* 336, 374-376

Guérin M, Barrois M, Terrier MJ, Spielman M, Riou G (1988) Overexpression of either c-*myc* or c-*erb*-B-2/*neu* proto-oncogenes in human breast carcinomas: correlation with poor prognosis. *Oncogene Res* 3, 21-31

Hancock JF, Magee AI, Childs JE, Marshall CJ (1989) All *ras* proteins are polyisoprenylated but only some are palmitoylated. *Cell* 57, 1167-1177

Hansen MF, Koufos A, Gallie BL, Phillips RA, Fodstad O, Brogger A, Gedde-Dahl T, Cavenee WK (1985) Osteosarcoma and retinoblastoma: a shared chromosomal mechanism revealing recessive predispositions. *Proc Natl Acad Sci USA* 82, 6216-6220

Harbour JW, Lai SL, Whang-Peng J, Gazdar AF, Minna JD, Kaye FJ (1988) Abnormalities in structure and expression of human retinoblastoma gene in SCLC. *Science* 241, 353-357

Herrera L, Kakati S, Gibas L, Pietrzak E, Sandberg AA (1986) Brief clinical report: Gardner syndrome in man with an interstitial deletion of 5q. *Am J Med Genet* 25, 473-476

Herskowitz I (1987) Functional inactivation of genes by dominant negative mutations. *Nature* 329, 219-222

Hicks GG, Mowat M (1988) Integration of Friend murine leukemia virus into both alleles of the *p53* oncogene in an erythroleukemic cell line. *J Virol* 62, 4752-4755

Hinds P, Finlay C, Levine AJ (1989) Mutation is required to activate the p53 gene for cooperation with the *ras* oncogene and transformation. *J Virol* 63, 739-746

Hong FD, Huang HJS, To H, Young LJS, Oro A, Bookstein R, Lee EYHP, Lee WH (1989) Structure of the human retinoblastoma gene. *Proc Natl Acad Sci USA* 86, 5502-5506

Huff V, Compton DA, Chao LY, Strong LC, Geiser CF, Saunders G (1988) Lack of linkage of familial Wilm's tumour to chromosomal band 11 p13. *Nature* 336, 377-378

Iguchi-Ariga SMM, Itani T, Kiji Y, Ariga H (1987) Possible function of the c-*myc* product: promotion of cellular DNA replication. *EMBO J* 6, 2365-2371

King CR, Swain SM, Porter L, Steinberg SM, Lippman ME, Gelman EM (1989) Heterogeneous expression of *erb*-B2 messenger RNA in human breast cancer. *Cancer Res* 49, 4185-4191

Kingston RE, Baldwin Jr AS, Sharp PA (1984) Regulation of heat shock protein 70 gene expression by c-*myc*. *Nature* 312, 280-282

Kitayama H, Sugimoto Y, Matsuzaki T, Ikawa Y, Noda M (1989) A *ras*-related gene with transformation suppressor activity. *Cell* 56, 77-84

Knudson Jr AG (1971) Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci USA* 68, 820-824

Lane DP, Crawford LV (1979) T antigen is bound to a host protein in SV40-transformed cells. *Nature* 278, 261-263

Lee WH, Bookstein R, Hong F, Young LJ, Shew JY, Lee EYHP (1987) Human retinoblastoma susceptibility gene: cloning, identification and sequence. *Science* 235, 1394-1399

Lee EYHP, To H, Shew JH, Bookstein R, Scully P, Lee WH (1988) Inactivation of the retinoblastoma susceptibility gene in human breast cancers. *Science* 241, 218-242

Leppert M, Dobbs M, Scambler P, O'Connell P, Nakamura Y, Stauffer D, Woodward S, Burt R, Hughes J, Gardner E, Lathrop M, Wasmuth J, Lalouel JM, White R (1987) The gene for familial polyposis coli maps to the long arm of chromosome 5. *Science* 238, 1411-1413

Ludlow JW, De Caprio JA, Huang CM, Lee WH, Paucha E, Livingston DH (1989) SV40 large T antigen binds preferentially to an underphosphorylated member of the retinoblastoma susceptibility gene product family. *Cell* 56, 57-65

Masuda H, Miller C, Kueffler HP, Battifora H, Cline MJ (1987) Rearrangement of the *p53* gene in human osteogenic sarcomas. *Proc Natl Acad Sci USA* 84, 7716-7719

Miller C, Mohandas T, Wolf D, Prokocimer MP, Rotter V, Koeffler HP (1986) Human *p53* gene localized to short arm of chromosome 17. *Nature* 319, 783-784

Morrison DK, Kaplan DR, Rapp U, Roberts TM (1988) Signal transduction from membrane to cytoplasm: growth factors and membrane-bound oncogene products increase c-*raf* phosphorylation and associated protein kinase activity. *Proc Natl Acad Sci USA* 85, 8855-8859

Murphree AL, Benedict WF (1984) Retinoblastoma: clues to human oncogenesis. *Science* 223, 1028-1033

Onclercq R, Gilardi P, Lavenu A, Cremisi C (1988) c-*myc* products *trans*-activate the adenovirus E4 promoter in EC stem cells by using the same target sequence as E1 A products. *J Virol* 62, 4533-4537

Parada LF, Land H, Weinberg RA, Wolf D, Potter V (1984) Co-operation between gene encoding p53 tumor antigen and *ras* in cellular transformation. *Nature* 312, 649-651

Pizon V, Chardin P, Lerosey I, Olofsson B, Tavitian A (1988) Human cDNAs *rap* 1 and *rap* 2 homologous to the Drosophila gene D*ras*3 encode proteins closely related to *ras* in the effector region. *Oncogene* 3, 201-204

Prendergast GC, Cole MD (1989) Post-transcriptional regulation of cellular gene expression by the c-*myc* oncogene. *Mol Cell Biol* 9, 124-134

Sarnow P, Ho YS, Williams J, Levine AJ (1982) Adenovirus E1b-58kd tumor antigen and SV40 T are physically associated with the same 54kd cellular protein in transformed cells. *Cell* 28, 387-394

Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. *Science* 235, 177-182

Sporn MB, Roberts AB (1988) Peptide growth factors are multifunctional. *Nature* 332, 217-219

Stanbridge EJ (1987) Genetic regulation of tumorigenic expression in somatic cell hybrids. *Adv Viral Oncol* 6, 83-101

T'Ang A, Varley JM, Chakraborty S, Murphree AL, Fung YKT (1988) Structural rearrangements of the retinoblastoma gene in human breast carcinoma. *Science* 242, 263-266

T'Ang A, Wu KJ, Hashimoto T, Liu WY, Takahashi R, Shi XH, Nihara K, Zhang FH, Chen YY, Du C, Qian J, Lin YG, Murphree AL, Qiu WR, Thompson T, Benedict WF, Fung YK (1989) Genomic organisation of the human retinoblastoma gene. *Oncogene* 4, 401-407

Tsuda H, Hirohashi S, Shimosato Y, Hirota T, Tsugane S, Yamamoto H, Niyajima N, Toyoshima K, Yamamoto T, Yokota J, Yoshida T, Sakamoto H, Terada M, Sugimura T (1989) Correlation between long-term survival in breast cancer patients and amplification of two putative oncogene-coamplification units: *hst*-1/*int*-2 and c-*erb*-B-2/*ear*-1. *Cancer Res* 49, 3104-3108

Veillette A, Brookman MA, Horak EM, Samelson LE, Bolen JB (1989) Signal transduction through the CD4 receptor involves the activation of the internal membrane tyrosine-protein kinase $p56^{lck}$. *Nature* 338, 257-259

Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AMM, Bos JL (1988) Genetic alterations during colorectal–tumor development. *N Engl J Med* 319, 525-532

Wasylyk C, Wasylyk B, Heidecker G, Huleihel M, Rapp UR (1989) Expression of *raf* oncogene activates the PEA1 transcription factor motif. *Mol Cell Biol* 9, 2247-2250

Whyte P, Buchkovich RJ, Horowitz JM, Friend SH, Raybuck M, Weinberg RA, Harlow E (1988) Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. *Nature* 334, 124-129

Wolf D, Rotter V (1985) Major deletions in the gene encoding the p53 tumor antigen cause lack of p53 expression in HL-60 cells. *Proc Natl Acad Sci USA* 82, 790-794

Wright C, Angus B, Nicholson S, Sainsbury JRC, Cairns J, Gullick WJ, Kelly P, Harris AL, Horne CHW (1989) Expression of c-*erb* B-2 oncoprotein: a prognostic indicator in human breast cancer. *Cancer Res* 49, 2087-2090