

Demystified ...

Oncogenes

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Oncogene research has been prolific over the past 10 years, producing more than 43 000 published articles. But what makes oncogenes so interesting? Probably the fact that they contribute to the death of 6.3 million patients with cancer worldwide each year.

This review will attempt to demystify oncogenes by describing the history of their discovery, how they are activated to become cancer causing molecules, their multitude of cellular functions, and their important clinical applications.

How were oncogenes discovered?

It is now taken for granted that cancer is a genetic disease, caused by mutations in a number of specific gene categories. However, Boveri's hypothesis in 1903—the first to speculate that cancer might result from chromosomal abnormalities—was not proven until relatively recently when, in 1960, cytogenetic analysis discovered the Philadelphia chromosome, an abnormality found consistently in the cells of patients with chronic myelogenous leukaemia (CML). This discovery was the first chromosomal anomaly associated specifically with a neoplasm, and therefore pointed to genetic material as the target of malfunction in a cancer cell. However, it was the study of tumour viruses that produced the first explicit example of the cancer causing genes we now know as oncogenes.

Viruses and cancer

A link between cancer and viruses had been made as early as 1909, when Peyton Rous discovered that a virus (now known as the Rous sarcoma virus) caused sarcomas in chickens. However, it took another 61 years before it was specifically shown that this virus used a single gene, the v-src oncogene, to switch an infected cell from normal to cancerous growth.¹

The Rous sarcoma virus is a member of the retrovirus family—viruses that possess an RNA genome encoding three genes essential for viral replication. However, the genome of the transforming Rous sarcoma virus contained a fourth gene, which was totally dispensable and had no effect on viral replication. Studies by Varmus and Bishop in 1976 resolved this puzzle when they showed the presence of src related sequences in the host (chicken) genome, concluding that this extra gene was actually not viral in origin, but had been accidentally picked up by the virus from its host, during a process termed transduction.² Transduction is possible

because, during replication, retroviruses copy their RNA genome into DNA, which is then reversibly integrated into the host genome (fig 1). Therefore, recombination between viral and host genomes can inadvertently lead to a gene being “kidnapped” and integrated into the viral genome. Moreover, src related sequences were also found in normal DNA sequences from a wide range of other vertebrates, including humans, suggesting that these genes were highly conserved in evolution.

The normal cellular genes, from which the retroviral oncogenes originated, became known as proto-oncogenes. Further studies showed that the cellular src proto-oncogene was not an anomaly, but rather served as an archetype, as more transduced oncogenes were identified in other transforming retroviruses (table 1).

Even though the link between retroviral infection and tumour development in animals was well established, few retroviruses had been implicated in human cancer. How then, if at all, were retroviral oncogenes linked to the pathogenesis of human tumours?

Oncogenes and human cancer

This question was answered by a series of very different studies³ in which DNA extracted from chemically transformed cells was shown to transform recipient mouse derived NIH-3T3 cells (fig 2). Subsequent genome analysis showed the presence of oncogenic sequences homologous to those found in the transforming retroviruses. Oncogenic sequences were subsequently identified in DNA extracted from both human tumour cell lines and biopsies.^{4,5} About 20% of human tumours tested in this way were shown to contain activated oncogenes.

A number of alternative strategies have been used to identify additional oncogenes involved in human tumorigenesis. Several oncogenes have been identified at the sites of chromosomal breakpoints characteristic of certain tumour types. For example, the abl oncogene was identified on chromosome 9 at the breakpoint of the Philadelphia chromosome in CML, involving the reciprocal translocation between chromosomes 9 and 22. Other oncogenes that have been shown to occur at chromosomal breakpoints include myc on chromosome 8q24 in Burkitt's lymphoma and bcl-2 on chromosome 18q21 in non-Hodgkin's lymphoma.

Oncogenes have also been identified in homogeneous staining regions and double minute chromosomes, two chromosomal

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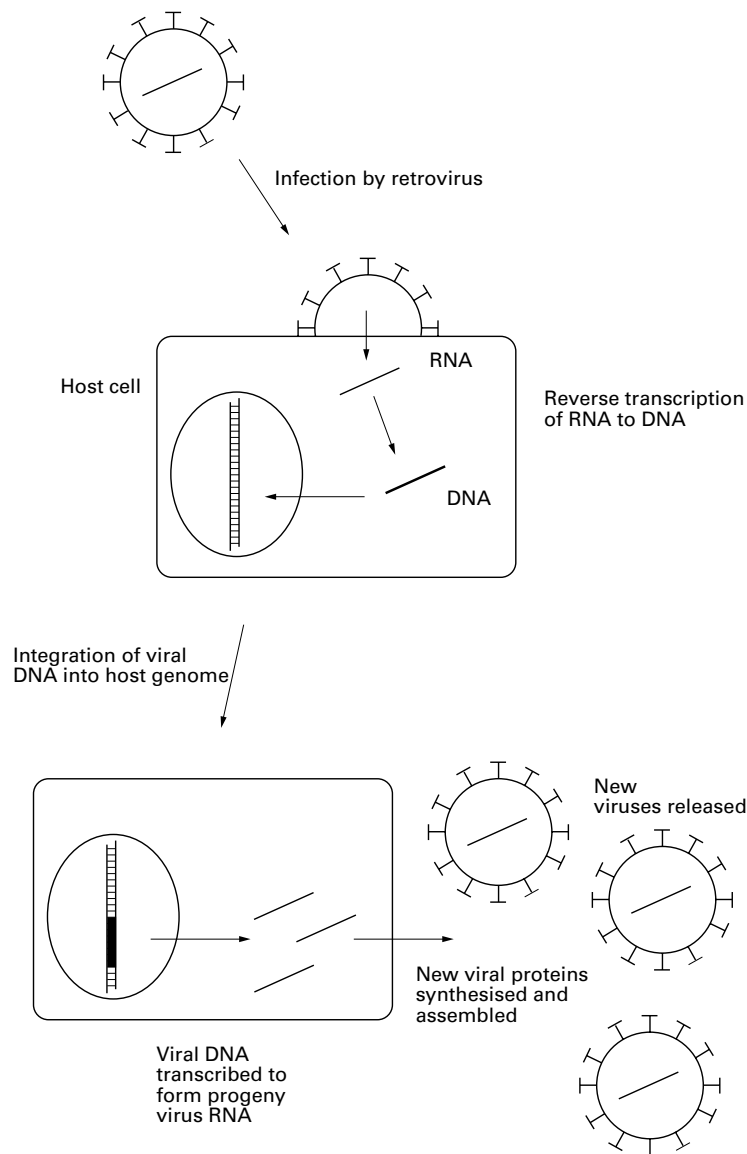


Figure 1 Retrovirus replication. After retrovirus infection of a host cell, a DNA copy of the viral RNA is synthesised by reverse transcription. The viral DNA is then integrated into the host genome and transcribed to produce progeny virus RNA.

abnormalities associated with oncogene amplification (see below). The genes N-myc associated with neuroblastomas and L-myc found in small cell lung carcinomas were first identified in this way.⁶ Nearly 200 proto-oncogenes have now been identified and an activated oncogenic

form of at least one of these genes has been shown to be associated with most human tumour groups.

Oncogene activation

As described above, proto-oncogenes are normal cellular genes, the products of which have been shown to be important components of intracellular signalling pathways (see below). The oncogenes, on the other hand, are not found in normal cells, but are generated by the activation of their corresponding proto-oncogenes during tumour development.

Activation of oncogenes results in a gain of function and may be quantitative (an increase in the production of an unaltered product) or qualitative (the production of a modified product). As a result of these alterations, activated oncogenes induce normal cell proliferation and therefore tumour development. Quantitative forms of oncogene activation occur either by amplification or by transposition to an active chromatin domain, whereas qualitative forms of activation occur either by point mutation or by the production of a novel product from a chimaeric gene.

ACTIVATION OF ONCOGENES BY AMPLIFICATION

Gene amplification results in increased gene expression and is common in tumour cells. Oncogene amplification may play a role in the progression of many tumours to more rapid growth patterns and increasing malignancy.

One of the best examples of oncogene amplification is the involvement of the N-myc gene in neuroblastoma as mentioned earlier. Amplified copies of N-myc are frequently present in late stage tumours and are therefore associated with the progression of neuroblastomas to increased levels of malignancy. Amplification of the erbB2 oncogene is a common finding in breast carcinoma. However, higher protein concentrations than would be predicted from the gene copy number alone in breast tumours suggest that other factors, in addition to gene amplification, are also involved. As mentioned in the previous section, the extra oncogene copies may be present either as small separate chromosomes (called double minutes) or as insertions within the normal chromosome—homogeneous staining regions.

ACTIVATION OF ONCOGENES BY TRANSPOSITION TO AN ACTIVE CHROMATIN DOMAIN

The overproduction of an oncogenic product may also occur by loss of transcriptional control through chromosomal translocation, as typified by the t(8;14) translocation seen in 75% of patients with Burkitt's lymphoma.⁷ Other patients exhibit translocations between chromosomes 8 and either chromosome 2 or 22. In all patients, the translocation causes the myc oncogene on chromosome 8 to become positioned next to an immunoglobulin gene: the heavy chain on chromosome 14; the κ light chain on chromosome 2; and the λ light chain on chromosome 22. The constitutive expression of the transposed myc gene after the

Table 1 Selected viral oncogenes and corresponding animals from which they were isolated

| Oncogene | Virus | Species |
|----------|-------------------------------|---------|
| abl | Abelson leukaemia | Mouse |
| erbB | Avian erythroblastosis ES4 | Chicken |
| ets | Avian erythroblastosis E26 | Chicken |
| fms | McDonough feline sarcoma | Cat |
| fos | FBJ murine osteogenic sarcoma | Mouse |
| jun | Avian sarcoma 17 | Chicken |
| mos | Moloney sarcoma | Mouse |
| myb | Avian myeloblastosis | Chicken |
| myc | Avian myelocytomatosis | Chicken |
| raf | 3611 murine sarcoma | Mouse |
| Ha-ras | Harvey sarcoma | Rat |
| Ki-ras | Kirsten sarcoma | Rat |
| sis | Simian sarcoma | Monkey |
| ski | Avian SK | Chicken |
| src | Rous sarcoma | Chicken |
| yes | Y73 sarcoma | Chicken |

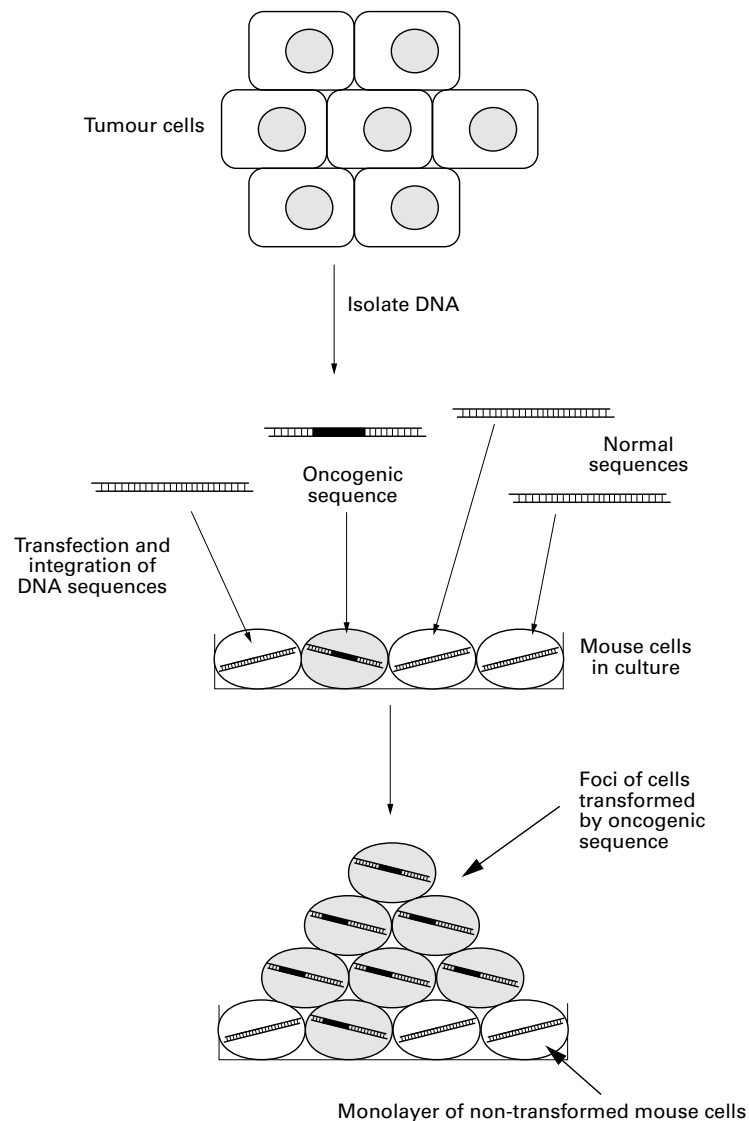


Figure 2 DNA transfection assay. DNA extracted from human tumour cells containing oncogenic sequences induces transformation of recipient mouse cells in culture, producing foci of transformed cells.

translocation thereby leads to an inappropriately high concentration of gene product.

ACTIVATION BY POINT MUTATION

Point mutations have been described in several oncogenes but have been studied most extensively in members of the ras family. Activating single base substitutions in these genes causing amino acid changes, particularly at positions 12, 13, and 61, have been detected in a wide range of human tumours, with an overall incidence of 10–15%, but as high as 95% in pancreatic carcinomas.^{8–10} These substitutions alter the structure of the normal protein, resulting in abnormal activity of the guanine nucleotide binding proteins that they encode (see below). Individual ras genes are commonly associated with specific tumours—for example, K-ras with cancers of the lung, colon, and pancreas and N-ras with acute myelogenous leukaemia. In addition, simultaneous mutations in all three ras members (K-ras, H-ras, N-ras) have been identified in thyroid adenomas and carcinomas.¹¹

ACTIVATION BY PRODUCTION OF CHIMAERIC GENE PRODUCTS

Oncogenes can also be activated by chromosomal translocation resulting in the production of a fusion protein. The best known tumour specific chromosomal rearrangement producing the small acrocentric Philadelphia chromosome is seen in 90% of patients with CML. This chromosome is produced by a balanced reciprocal 9;22 translocation. As already touched upon, the breakpoint on chromosome 9 lies within an intron of the abl proto-oncogene. The translocation joins most of the abl genomic sequence on to a gene called bcl (breakpoint cluster region) on chromosome 22, thereby creating a novel fusion gene. The fusion of the bcl sequence to the N-terminus of the abl gene results in both aberrant activity and subcellular location of the Abl protein tyrosine kinase, thereby leading to cell transformation.

Normal cell proliferation is regulated by biochemical pathways activated by growth factors interacting with their receptors on the plasma membrane, eventually leading to alterations in gene expression. Proto-oncogene protein products have been identified that function at all of the known steps involved in these signalling pathways.

Activation of a proto-oncogene (by one of the mechanisms discussed above) to the corresponding oncogene will therefore predominantly contribute to the abnormal regulation of cell proliferation seen in tumour cells. In addition, some oncogene products contribute to other aspects of cancer cell behaviour such as defective differentiation and failure to undergo programmed cell death or apoptosis. The next section will describe some of the most important functions of oncogene products.

Functions of oncogene products

As already alluded to, most oncogene protein products function as elements of the signalling pathways that regulate cell proliferation in response to growth factor stimulation. These products include growth factors, growth factor receptors, signal transducers, and transcription factors (fig 3). Oncogene functions also involve the direct control of the cell cycle and the inhibition of apoptosis.

ONCOGENES AS GROWTH FACTORS

Growth factors include a wide variety of signalling molecules that initiate the control of cell growth and proliferation. The action of these polypeptide factors as oncogenic proteins results from their abnormal expression leading either to autocrine stimulation (in which the tumour cell secretes and responds to the growth factor) or paracrine stimulation (in which the tumour cell responds to growth factors secreted by neighbouring stromal cells). The autocrine model has been proposed for the way bombesin secretion acts in small cell lung cancer. These tumour cells produce large amounts of the peptide, which causes the hydrolysis of the membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP₂), thereby leading to an increase in intracellular

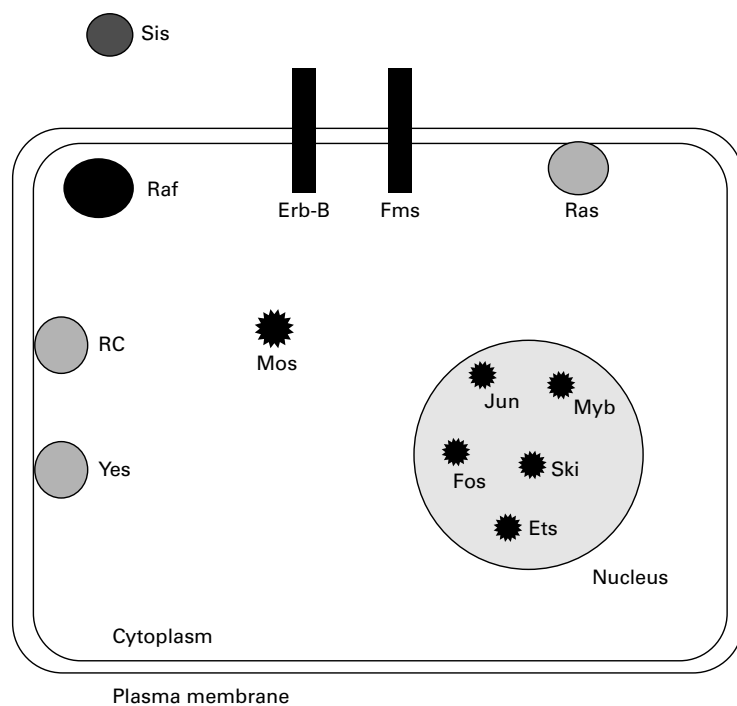


Figure 3 Examples of cellular locations of a number of oncogene protein products, including growth factors (for example, Sis), growth factor receptors (for example, ErbB), signal transducers (for example, Raf), and transcription factors (for example, Fos). Adapted from ref 12 with permission from Bios Scientific Publishers.

calcium, a characteristic response of cells entering the cell cycle.

ONCOGENES AS GROWTH FACTOR RECEPTORS

A large number of oncogenes encode growth factor receptors, which link the information from the extracellular environment (growth factors) to a number of different intracellular signalling pathways. Most of this group of oncogenes encode members of the transmembrane receptor protein tyrosine kinase family which, as their name suggests, phosphorylate their substrates on tyrosine residues and comprise the most important group of growth factor receptors with respect to malignant transformation. These receptors possess an N-terminal extracellular ligand binding domain, a single transmembrane α helix, and a cytosolic C-terminal domain with kinase activity. The most characteristic feature of these receptors is that upon ligand binding they form dimers, which leads to autophosphorylation of the receptor. Receptor phosphorylation then enables the phosphorylation of target proteins required to propagate the signal initiated by growth factor binding.

These receptors are frequently converted to oncogenic proteins by deletion of their N-terminal ligand binding domains, which causes constitutive activation of the kinase domain and leads to upregulation of the proliferative signal. This is exemplified by the *erbB1* oncogene, which encodes the epidermal growth factor (EGF) receptor, in which deletion of amino acids 6–273 is frequently detected in brain, lung, breast, and ovarian tumours.¹⁵ Alternatively, genes that encode some receptor protein tyrosine kinases are acti-

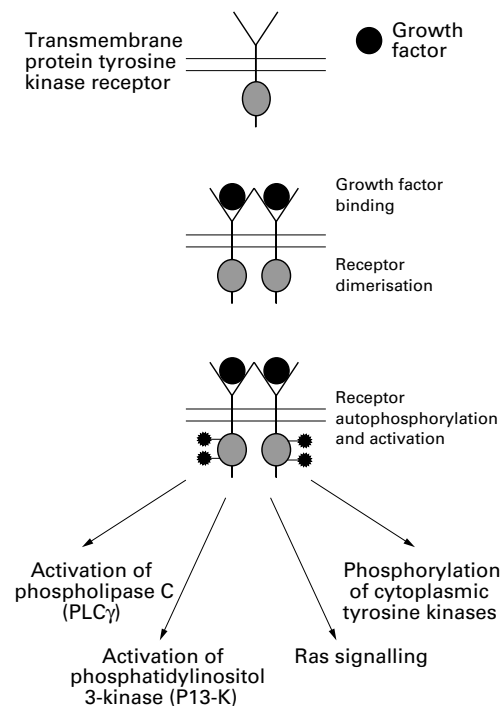


Figure 4 Growth factor binding at the cell surface produces dimerisation of transmembrane protein tyrosine kinase receptors. Activation of these receptors leads to a number of downstream signalling pathways. Adapted from ref 12 with permission from Bios Scientific Publishers limited.

vated by gene amplification, as exemplified by *erbB2*, which is amplified up to 50-fold in about 20% of bladder tumours.¹⁴

ONCOGENES AS SIGNAL TRANSDUCERS

The autophosphorylation of transmembrane receptor protein tyrosine kinases facilitates the binding of signalling molecules to the receptors. This is essential for the transmission of signals initiated by growth factor binding at the cell surface. Signalling molecules associate with phosphorylated receptors via their SH2 domains (SRC homology 2), which consist of ~100 amino acids and bind to specific short peptide sequences containing phosphotyrosine residues. The binding of signal transducers to activated receptors leads to a number of downstream signalling pathways (fig 4).

As shown in fig 4, one pathway leads to the activation of the enzyme phospholipase $C\gamma$ ($PLC\gamma$), which catalyses the hydrolysis of PIP_2 to yield the second messengers diacylglycerol (DAG) and inositol triphosphate (IP_3). A second pathway activates the enzyme phosphatidylinositol 3-kinase (PI3-K), which phosphorylates PIP_2 , producing the distinct second messenger PIP_3 (phosphatidylinositol 3,4,5-triphosphate). Both $PLC\gamma$ and PI3-K are activated by binding to activated transmembrane receptor protein tyrosine kinases via their SH2 domains.

The Ras signalling system is particularly important with respect to malignant transformation because it contains molecules with important oncogenic roles in human tumorigenesis. The Ras proteins play a key role in mitogenic signalling by coupling growth factor

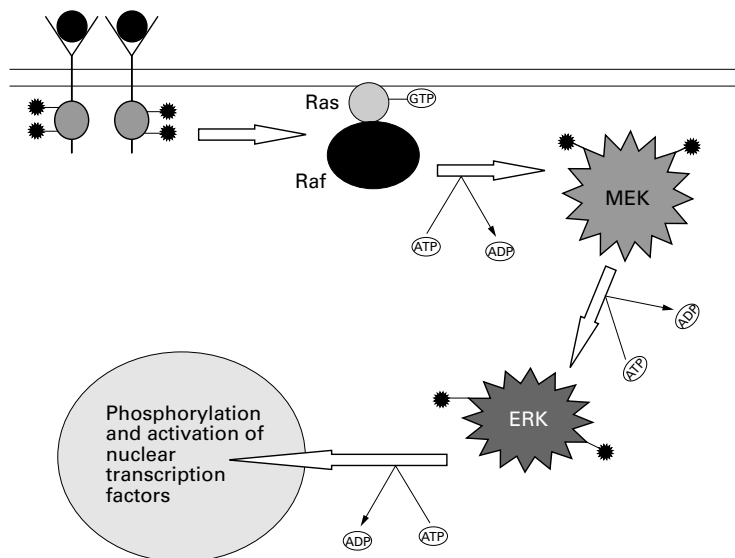


Figure 5 Schematic diagram to show activation of the ERK MAP kinases. Growth factor binding leads to activation of Ras protein (fig 4), which causes activation of Raf. Raf phosphorylates and activates MEK (MAP kinase/ERK kinase). MEK activates ERK by phosphorylation, leading to phosphorylation and activation of a number of nuclear transcription factors.

receptors to activation of the Raf1 protein serine/threonine kinases, which then initiate a protein kinase cascade leading to the activation of the ERK MAP kinases (extracellular signal regulated kinase mitogen activated protein kinases). These cascades ultimately lead to phosphorylation of nuclear transcription factors and therefore altered gene expression, as illustrated schematically in fig 5.

The ras genes (K-ras, H-ras, and N-ras) each encode a 21 kDa protein (p21), which is a guanine nucleotide binding protein that alternates between an inactive GDP bound form and an active GTP bound form. Ras activation is mediated by guanine nucleotide exchange factors that stimulate the exchange of GDP for GTP. Activity of the Ras-GTP complex is terminated by GTP hydrolysis by GTPase activating proteins (GAPs). The mutations that convert ras proto-oncogenes to oncogenes result in decreased GTPase activity, thereby leading to constitutive Ras activity and therefore activation of the MAP kinase pathway. High levels of raf1 gene expression have also been shown to occur in some small cell lung cancers, which would also lead to increased MAP kinase activity.¹⁵

The product of the src oncogene is a member of the cytoplasmic tyrosine kinase signal transducers shown both to be activated by a variety of transmembrane receptor protein tyrosine kinases and to have oncogenic potential. Recent studies have shown that active Src is essential for the stimulation of DNA synthesis in response to platelet derived growth factor (PDGF) and that Src kinases may also control transcription of the Myc transcription factors.¹⁶ Mutations have not been detected directly in the src gene; however, increased Src kinase activity has been reported in some colon cancers, skin tumours, and breast carcinomas.¹⁷⁻¹⁹

ONCOGENES AS TRANSCRIPTION FACTORS

Many proto-oncogenes encode transcription factors that are normally induced in response to growth factor stimulation. These proteins regulate the expression of growth control genes by binding to specific DNA sequences. The transcription factors fall into two broad categories: those that only interact with DNA as complexes with other proteins—for example, Myc; and those that in monomeric form possess a high affinity for specific DNA sequences—for example, Myb. The activity of these transcription factors is regulated by phosphorylation.

The Myc proteins (Myc, N-Myc, and L-Myc) all contain basic, helix-loop-helix and leucine zipper domains and form heterodimers with the protein Max.²⁰ A leucine zipper domain is composed of leucine residues exposed on one side of a helical region of a polypeptide chain. Interactions between the hydrophobic side chains of these leucine residues facilitate polypeptide chain dimerisation. Myc-Max heterodimers function as sequence specific transcriptional regulators and dimerisation with Max is essential for DNA binding. Max also forms heterodimers with other leucine zipper proteins, including Mad and Mx11. These heterodimers bind to the same DNA sequences as the Myc-Max heterodimers and will therefore repress transcription activation by Myc-Max complexes.

The principal functions of the myc gene products are the induction of cell proliferation and the inhibition of terminal differentiation in response to mitogenic stimuli. These genes are converted to oncogenes by either amplification and/or overexpression, which occurs commonly in a wide range of human tumours. N-myc is amplified frequently in neuroblastomas, retinoblastomas, gliomas, and astrocytomas, whereas amplification of L-myc has been detected in small cell lung carcinomas.²¹

The transcription factor Myb is involved in controlling cell cycle progression and differentiation. The Myb protein contains transcriptional activation, negative regulatory, and sequence specific DNA binding domains. Myb positively regulates its own transcription as well as stimulating the transcription of a large number of genes, including myc. Again, like myc, myb is usually activated by amplification, which has been detected in a number of leukaemias, colon carcinomas, melanomas, breast cancers, and also in some ovarian and cervical tumours.²²⁻²⁶

ONCOGENES AS CELL CYCLE REGULATORS:

CYCLIN D1

The intracellular signalling pathways activated by growth factor stimulation ultimately regulate components of the cell cycle machinery that promote progression through the restriction point in G1. The D-type cyclins are induced in response to growth factor stimulation and play a key role in coupling growth factor signalling to cell cycle progression. Not surprisingly, genes encoding these proteins have been shown to exhibit oncogenic potential.

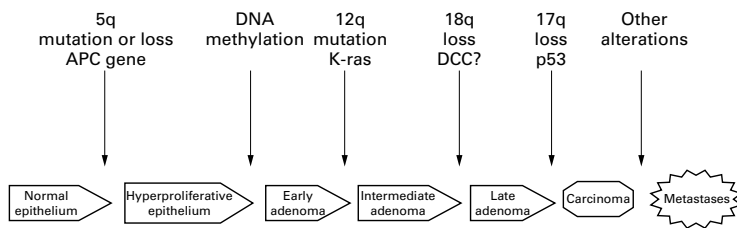


Figure 6 Genetic model of colorectal tumorigenesis illustrating the molecular events involved in the progression of normal colonic epithelium to metastases by the adenoma-carcinoma sequence.

Cyclin D1 is expressed in a cell cycle specific manner. In a complex with one of its cyclin dependent kinases (CDK4/6) it phosphorylates the retinoblastoma protein (pRb) before the S phase of the cell cycle. Hyperphosphorylation of pRb disrupts E2F-pRb complexes, releasing the E2F transcription factor and therefore allowing it to activate the transcription of genes necessary for S phase entry.

The gene encoding cyclin D1 is a proto-oncogene that can be activated to an oncogene (called PRAD1) by gene amplification, thereby leading to constitutive expression and driving the cell cycle forward beyond G1 in the absence of normal growth factor stimulation. PRAD1 amplification has been detected in some gastric, breast, and oesophageal cancers.^{27 28} Overproduction of cyclin D1 has also been shown to result from a t(11;14) chromosomal translocation in some B cell non-Hodgkin's lymphomas, B cell chronic lymphocytic leukaemias, and multiple myelomas.²⁹

ONCOGENES AS APOPTOSIS INHIBITORS

The failure of some cancer cells to undergo apoptosis (programmed cell death) is an important factor in tumour development, as exemplified by the bcl-2 oncogene, which appears to contribute to the development of some lymphomas by protecting against apoptosis rather than by stimulating cell proliferation. The bcl-2 oncogene in these cancers is generated by the chromosomal translocation t(14;18)(q32;q21), which is a specific abnormality of human lymphoid neoplasms occurring in 85% of follicular lymphomas and in 20% of diffuse lymphomas. The translocation involves the immunoglobulin heavy chain locus at chromosome 14q32 and the bcl-2 gene on chromosome 18q21, and results in increased bcl-2 expression. Because the normal function of bcl-2 is to suppress apoptosis, its increased expression will reduce levels of apoptosis, thereby maintaining cell survival, and contributing to both tumour formation and progression.

Clinical application of oncogenes

Most cancers have now been examined for the presence or absence of activated oncogenes and at least one has been found to be abnormally expressed in most tumour types. In most cases, there is little direct clinical application for these findings, the presence of an activated oncogene being the result of the general destabilisation of the cell as it has become

malignant. However, there are some notable exceptions and some of the more useful, but by no means exhaustive, clinical applications are discussed below.

Colorectal cancer

Alterations in ras have been studied extensively in colorectal adenomas and cancers. Many (37–60%) colorectal tumours carry point mutations in the K-ras gene, primarily at codons 12, 13, or 61.³⁰ Mutations are also found in ~50% of adenomas, suggesting that these changes are an early event in the development of malignancy. However, mutations are rarely found in adenomas smaller than 1 cm in size,³⁰ which has led to the suggestion that point mutations in ras may be required for the conversion of small adenomas to large ones by clonal expansion of the cells carrying the mutation. Mutations in ras have also been found in grossly normal colorectal mucosa and subsequently shown to be present in aberrant crypts within the grossly normal mucosa.³¹

No correlations have been found between the presence of a ras mutation and five year survival.^{32 33} Two studies have looked at the nature of the amino acid substitution at codon 12 of K-ras with conflicting findings. In the first study, metastases were almost completely associated with the substitution of glycine with aspartic acid³⁴ whereas, in the second study, this alteration was found in Duke's B tumours and metastases were either associated with glycine to valine or glycine to alanine changes.³⁵

Because ras mutations are a fairly early event in the development of colorectal tumours, several studies have looked for these mutations in stool samples, with the aim of using this as a screening test to detect cancers at an early stage.^{36 37} The potential value of the technique was highlighted by the detection of ras mutations in the stools of several patients who, when investigated by colonoscopy, had only adenomas present.³⁶ The ultimate potential of this technology still remains to be evaluated but it may eventually be superseded by the identification of mutations in the tumour suppressor gene, APC.³⁸ Mutations in this gene are known to be the initiating mutations in the development of colorectal cancer (fig 6). However, the variety and number of different mutations in APC, together with the technology currently available, means that it is impossible to use this gene at present as part of a screening programme for sporadic disease.

Gastric cancer

Gastric cancer has been associated with a number of alterations in growth factors and their receptors. In particular, overexpression of transforming growth factor β (TGF- β), insulin-like growth factor (IGF), and PDGF have all been found, particularly in association with poorly differentiated and scirrous cancers.³⁹ A novel member of the EGF family, cripto, is overexpressed in 35% of gastric cancers, and a good correlation was found between expression and tumour stage and prognosis.³⁹ Overexpression of the EGF receptor is found in

association with well differentiated tumours and has been associated with poor prognosis.⁴⁰

Pancreatic cancer

Mutations of the ras gene have been found in up to 85% of pancreatic cancers, almost exclusively at codon 12 of K-ras. As with colorectal cancer, ras mutations have also been found in the stools of pancreatic cancer patients, suggesting that it might be a useful screening tool. However, the pick up rate of mutations of just over 50%, and the identification of mutations in the stools of patients with pancreatitis and no confirmed cancer, clearly limits the value of this technique for screening.⁴¹

Breast cancer

RAS

Increased expression of ras has been detected immunohistochemically in 63–83% of malignant breast tumours. Two studies have shown an association between high concentrations of Ras and a short disease free interval and with progression and poor prognosis.⁴² An association between cancer and the presence of rare alleles of a polymorphism located 1000 base pairs downstream from the coding region of H-ras has been known for over 10 years.⁴³ Meta-analysis has now shown that women heterozygous for one of the rare alleles have a 1.7-fold increased risk of breast cancer, whereas those homozygous for the rare allele have a 4.6-fold increased risk for a common cancer, including breast cancer.⁴⁴

erbB2

erbB2 amplification has been found in 10–30% of breast cancers. Several studies have shown that amplification is associated with poor prognosis. Initially, this was only seen in lymph node positive patients but subsequently lymph node negative patients were also shown to have a worse prognosis when erbB2 was amplified.⁴⁵

Lung cancer

MYC

Abnormal expression of all three myc genes has been detected in both small cell and non-small cell lung cancer. Amplification of myc, resulting in overproduction of the p62 protein product, has been associated with progression of small cell lung cancer.^{46 47} Small cell lung cancer tumours showing amplification of myc have been suggested to be more aggressive and patients with these tumours have a worse prognosis.⁴⁸ Increased expression of N-myc has been correlated with a poor response to chemotherapy, rapid tumour growth, and short survival times.⁴⁸ A restriction fragment length polymorphism (RFLP) associated with the L-myc gene has been implicated in the prognosis of a number of tumours. In non-small cell lung cancer, those patients with the 6 kb allele of the RFLP, whether in the heterozygous or homozygous state, had the highest number of lymph node metastases as well as an increased incidence of metastases to other organs.⁴⁷

RAS

Patients with small cell lung cancer and K-ras codon 12 mutations have a significantly poorer survival than those without such mutations.⁴⁹ Carcinogens in cigarette smoke have been implicated as the cause of ras point mutations in non-small cell lung cancer.⁴⁸ As in breast cancer, rare polymorphic alleles of H-ras are associated with the more aggressive non-small cell lung cancers rather than with small cell lung cancers or individuals without lung cancer.⁵⁰

Genitourinary cancers

MYC

Amplification and overexpression of myc have been associated with advanced tumours of the uterine cervix. Patients showing overexpression of myc have been shown to be at an eightfold increased risk of relapse, a feature which outweighs nodal as a prognostic factor.⁴²

Overproduction of the p62 protein product of myc has been correlated with increasing differentiation of testicular teratomas. Patients with no recurrence three years after diagnosis showed a significantly higher concentration of p62 than those who developed a recurrence within the same time period.⁵¹

RAS

Synthesis of the Ras p21 protein, as determined by immunohistochemical investigations, is associated with high grade tumours of the prostate and in one study has been shown to be the only marker associated with tumour grade.

Neurological tumours

N-myc

Neuroblastomas are the commonest childhood tumour, with a mean age at diagnosis of 30 months for sporadic cases and 9 months for those with a familial predisposition. Cytogenetic studies identified the presence of double minute chromosomes and homogenous staining regions in neuroblastomas. These were subsequently shown to be regions of N-myc amplification. This oncogene was one of the first oncogenes found to be of use clinically. Stage III and IV neuroblastomas have a poor prognosis with only 10–30% survival at two years. Both these stages of tumour show amplification of N-myc. By contrast, N-myc amplification is rarely associated with stage IV tumours in which regression has occurred.⁵²

Haematological malignancies

ABL

The 9;22 translocation is found in most patients with CML, in adult and childhood acute lymphocytic leukaemia, and occasionally in acute myeloid leukaemia. This chromosomal rearrangement can be detected cytogenetically in most cases, but can also be picked up at the molecular level in the absence of an obvious chromosomal translocation. This is important because patients without the rearrangement have a worse prognosis. Because the rearrangement can be detected using the polymerase chain reaction at the level of one malignant cell in a population of 10^5 – 10^6 cells, it is possible to

monitor patients accurately after bone marrow transplantation to detect residual disease and permit early treatment to prevent relapse, thereby helping to sustain remission.

Multiple endocrine neoplasia 2 (MEN2)

The gene for MEN2 was assigned to chromosome 10 in 1987. Unlike other familial cancer genes there was no evidence of loss of heterozygosity (LOH) in the region: LOH usually being a feature associated with the involvement of a tumour suppressor gene. This suggested that the causative gene was more likely to be a dominantly acting gene. The ret proto-oncogene, the product of which is a receptor tyrosine kinase associated with transduction of signals for cell proliferation, also mapped to the same region of chromosome 10. It was quickly shown that activating mutations in ret were found in patients with MEN2A and MEN2B, as well as familial medullary thyroid cancer.⁵³ MEN2A and familial medullary thyroid cancer are associated with mutations in the region of the gene that encodes the cysteine extracellular domain of the receptor, with most mutations resulting in the replacement of a cysteine amino acid by another amino acid. Over 70% of mutations in MEN2A and familial medullary thyroid cancer occur at codon 634. In MEN2B the causative mutation in most cases is a methionine to threonine substitution at codon 918.⁵⁴

The identification of ret gene mutations in patients with MEN and familial medullary thyroid cancer means that molecular testing can be used to identify family members at high risk of developing the disease. Those individuals at low risk then no longer have to undergo an unpleasant as well as costly screening programme. Patients identified to be at high risk can then be offered total thyroidectomy to prevent the development of thyroid cancers.

Hirschsprung disease

Hirschsprung disease is an abnormality of the hindgut characterised by the absence of enteric autonomic ganglia but it is also associated with mutations in the ret gene. In contrast to MEN2, where mutations result in a gain of function of the ret gene product, Hirschsprung disease arises because of mutations leading to a loss of function.⁵⁵ Inactivating mutations are scattered throughout the extracellular and intracellular domains of ret.

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