

this eliminates differences between the sexes and between young and old.¹⁵ The mean VC in the white subjects was significantly higher than in subjects from the other three groups. Consequently, when the ventilatory response was corrected for these differences in VC, the minor differences still present just failed to reach statistical significance. Occlusion pressure measurements would, however, have avoided the problems related to lung volumes.^{16,17} Furthermore, as we have only studied small groups of patients, larger studies would be needed to avoid making the type 2 error of claiming lack of significance.

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Cancer — approaching a universal molecular mechanism?

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Summary

Accumulating evidence strongly suggests that cancer is a genetic disease, arising from mutations in DNA. These mutations alter the function or synthesis of two groups of proteins, which are the products of either proto-oncogenes or anti-oncogenes. Of the more than 30 proto-oncogenes identified, *ras* proto-oncogenes are most frequently found to be mutationally activated (to oncogenes) in human tumours. Developments leading to current understanding of the function of *ras* proto-oncogenes and of the retinoblastoma anti-oncogene are reviewed. Based on the involvement of all known oncogenes and anti-oncogenes in cellular signal transduction pathways, it is suggested that a general model for cancer at the molecular level may become a reality.

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The term 'cancer' indicates any of the various types of malignant neoplasms; cancer cells are characterised by loss of normal growth control and the ability to metastasise. Accumulating evidence suggests that cancer is a genetic disease; mutations in DNA affecting the expression of certain genes are almost certainly necessary for the transition of a normal cell to tumorigenicity. These mutations ultimately alter the activity of proteins involved in cellular chemical communication. The rapid advances being made in understanding these complex communication pathways and their role in cellular transformation is set to change our understanding and treatment of cancer.

Proto-oncogenes are normal cellular genes. They code for the synthesis of certain proteins (proto-oncoproteins or oncoproteins), all of which appear to be important in the communication of extracellular signals to the cell nucleus. However, certain mutations or rearrangements can cause these proteins to be modified in function or to be produced in inappropriate amounts, thereby contributing to cell transformation and tumour development.

Some oncogenes were initially identified as the transforming genes of animal tumour retroviruses. These genes were acquired by transduction and mutation of normal cellular proto-oncogenes from the host cell (Fig. 1). For example, the cellular homologue of the *v-sis* oncogene is platelet-derived growth

ONCOGENE	ORIGINAL VIRAL HOST	CELLULAR HOMOLOGUE	ROLE	CELLULAR LOCALISATION
sis	Simian monkey	PDGF A-chain	Secreted growth	Extra-cellular
erbB	Chicken	EGF receptor	Receptor (tyrosine kinase)	Cell membrane
H-ras	Rat	c-H-ras	G-protein signal transducer?	Inner cell membrane
mos	Mouse	c-mos	Protein phosphorylation	Cytoplasm
myc	Chicken	c-myc	Control of cell division	Nucleus

Fig. 1. Combined table summarising some characteristics of a selection of oncogenes, with a schematic diagram of a cell illustrating the subcellular localisation of their protein products.

factor (PDGF), and the *v-erbB* oncogene was transduced from the receptor for epidermal growth factor (EGF).

More than 30 oncogenes have been identified from viruses, tumours or both. In addition, considerable progress has been made in identifying and understanding the role of anti-oncogenes or tumour-suppressor genes in cancer. Some information pertaining to both a classic oncogene and an anti-oncogene is presented and discussed in terms of a potential model for cancer development at the molecular level.

The T24 bladder carcinoma and *ras* oncogenes

Transfection of DNA isolated from the human bladder carcinoma cell line (T24) into established rodent cells (NIH 3T3 cells) in culture induced a small number of these cells to become tumorigenic.^{1,2} A mutated human *H-ras* gene in the carcinoma DNA was responsible for the transforming activity. A point mutation changing the normally encoded glycine at amino acid 12 to a valine was responsible for the transforming activity of the oncogene.³ Furthermore, the human *H-ras* gene (*c-H-ras1*) had a counterpart in the transforming gene of the Harvey murine sarcoma virus (*v-H-ras*);⁴ this encodes an arginine at amino acid 12, which contributes to its transformation potential.^{4,5}

There are three human *ras* proto-oncogenes with very similar protein products. *H-* and *K-ras* have counterparts in murine Harvey and Kirsten sarcoma viruses, while *N-ras*, identified as an oncogene in a human neuroblastoma, has no known viral equivalent.⁶ The three *ras* proto-oncogenes are normal cellular genes encoding proteins that are important in cell growth and differentiation. Mutations altering one of a few crucial amino acids (e.g. the glycine at position 12) change a *ras* proto-oncogene to an oncogene. Activating mutations of *ras* genes are very common in carcinogen-induced tumours in animals.² These mutations alter the activity of the *ras* protein (p21) enabling it to transform rodent cells in culture. This provides a useful assay for transforming oncogenes. It is clear that activating mutations of *ras* genes contribute to the development of many human tumours.²

Sophisticated techniques have been used to compare DNA from tumour and normal cells of patients in order to detect the presence of activating mutations in *ras* genes. About 25% of human tumour DNAs analysed harbour such mutations. Analyses have proved that more than 40% of human colon and thyroid carcinomas have activating *ras* gene mutations.⁷⁻⁹ Investigations of over-expressed normal p21 in tumours are frequently published,^{10,11} but these high expression levels are as likely to be a consequence of the tumour condition as a cause.¹²

The *ras* proto-oncogenes code for a protein (called p21 because its molecular weight approximates 21 000 daltons) that is localised to the inner surface of the cell membrane (Fig. 1 and Fig. 2). The protein p21 binds guanosine triphosphate (GTP) and hydrolyses it to guanosine diphosphate (GDP). These characteristics suggest that it functions as a GTP-binding protein, mediating signal transduction between membrane receptors and intracellular effector proteins. However, since no receptor or effector has been found that clearly associates with p21, this remains only a plausible hypothesis. Investigations have identified a protein (termed GAP) that stimulates the GTPase activity of p21 but, as yet, this has no clear effector function.¹³ Elucidation of the normal function of *ras* p21 is clearly important for a better understanding of cancer at the molecular level.

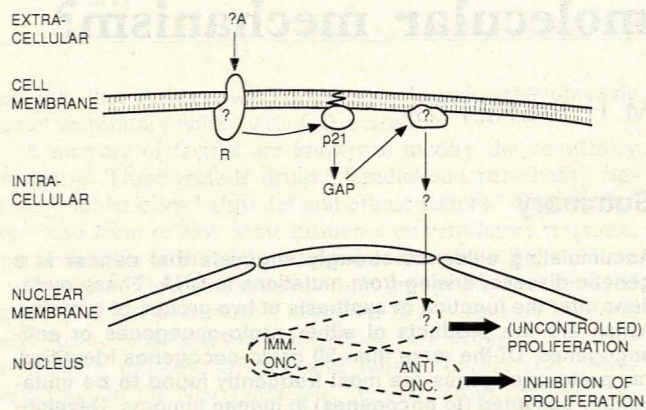


Fig. 2. Hypothetical scheme of $p21^{ras}$ function in signal transduction illustrating the proposed functional link between $p21^{ras}$ and immortalising oncogenes (such as $pp110^{Rb}$) (?A = unknown agonist; ?R = unknown receptor; GAP = GTPase activating protein; Imm. Onc. = immortalising oncogene; Anti-Onc. = anti-oncogene; ? = unidentified hypothetical intermediates).

Activation of a *ras* gene alone is insufficient to convert a normal cell to tumorigenicity. Introducing (by DNA transfection) cloned DNA coding for an activated *ras* gene into primary cells is not sufficient to achieve transformation. Other oncogenes, such as *myc* or adenovirus E1A, must also be expressed and then collaborate with *ras* to transform primary cells. In addition, the loss of heterozygosity found in many *ras*-transformed cell lines indicates that loss of expression of anti-oncogenes is also important in tumour development.²

The retinoblastoma gene is an anti-oncogene

With an ophthalmoscope, the ability to diagnose and timeously treat retinoblastoma (RB) led to its emergence and recognition as a hereditary disease.¹⁴ In 1971 Knudson¹⁵ proposed that two genetic events were necessary for the development of RB; in the hereditary form one of these events is inherited through a parental germ-line.

Cytogenetic studies¹⁶ showed a specific region of chromosome 13 (band q14) to be deleted in many RB cells. Using DNA cloning techniques, including 'chromosome walking', a candidate gene at 13q14 was found; the gene was expressed in normal retinal cells, but not in RB cells.¹⁷

Furthermore, chromosomal deletions involving parts of this gene occurred in a number of RBs. Knudson's¹⁵ hypothesis was thus essentially correct. The two mutational events leading to the development of RB are mutations resulting in loss of expression from both alleles of the RB gene. RB therefore arises in retinal cells unable to synthesise any normal protein from the RB gene. Confirming this, tumorigenic RB cells in culture become non-tumorigenic when an active RB gene is introduced into the cells. The reverted cells synthesise the protein product of the RB gene (pp110^{RB}).¹⁸

The RB gene is therefore an anti-oncogene. Although surgically treated patients with the hereditary form of RB were known to be predisposed to osteosarcoma, inactivating mutations of the RB gene have now been found in many tumours besides RBs and osteosarcomas.¹⁸ This clearly indicates that knowledge of the RB gene and its protein product (pp110^{RB}) has a bearing on more than just the relatively rare RB.

Although the prototype anti-oncogene, the RB gene is certainly not the only such gene. A nuclear protein, termed p53, appears to also act as an anti-oncogene.¹⁹ In addition, hereditary Wilms' tumour (a nephroblastoma) is almost certainly caused by loss of expression of an as yet unidentified anti-oncogene located on chromosome 11 (band p13).²⁰

Other oncogenes, *ras* and RB

The RB gene codes for a protein (pp110^{RB}) that localises to the nucleus of the cell. The protein pp110^{RB} could limit the cellular proliferation characteristic of tumour cells by binding to and inactivating proteins in the cell nucleus that stimulate cell growth and division. The different subcellular locations of *ras* p21 and pp110^{RB} preclude a direct interaction of the two proteins. However, pp110^{RB} does bind the transforming nuclear oncoproteins of certain DNA viruses, such as adenovirus E1A, SV-40 large T-antigen and HPV-16 E7 protein.²¹ These viral proteins function to stimulate DNA synthesis in their host cells, thereby facilitating their own replication.

The DNA virus oncogenes described above function as immortalising oncogenes. Their expression can immortalise primary cells but not transform them to tumorigenicity. The human *c-myc* oncogene, best known for its re-arrangement and over-expression in Burkitt's lymphoma, is also an immortalising oncogene. Expression of activated *ras* cannot immortalise primary cells, and consequently does not transform them. However, co-expression of an immortalising oncogene (such as *c-myc*) and activated *ras* will transform primary cells to tumorigenicity. The region of the adenovirus E1A oncoprotein necessary for binding pp110^{RB} is present in other immortalising oncoproteins, including *c-myc*. The protein products of immortalising oncogenes may therefore act by neutralising anti-oncoproteins, thereby facilitating transformation by other oncogenes, such as *ras*. The immortalising oncoproteins therefore provide a functional link between the membrane-attached oncoprotein, p21, and the nuclear anti-oncoprotein, pp110^{RB} (Fig. 2).

Concluding remarks

Cancer is a genetic disease; mutations in cellular DNA affecting certain genes normally involved in control of cell growth permit a normal cell to become cancerous. A link between the two very diverse proteins of this group, namely the putative signal transducing, membrane-bound oncoprotein (p21^{ras}), and the nuclear anti-oncoprotein (pp110^{RB}), through the 'immortalising oncogenes' has been outlined. These two proteins are of central importance to the development of a large proportion of all human tumours.

It is suggested that a network of interactions involving proto-oncoproteins in normal signal transduction and in cancer can be envisaged. Hypothetical transformation pathway schemes have already been proposed and serve as working models for further research.^{22,23} However, the complexity of signal transduction pathways and the different use of intermediates in such pathways by various cell types must be considered. None the less, schematic diagrams of signal transduction pathways, analogous to the metabolic pathways of old, indicating the sites at which aberrant expression or function can contribute to tumorigenicity, may not be far off.

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