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# Cellular oncogenes in neoplasia

V T W CHAN, J O'D McGEE

*From the University of Oxford, Nuffield Department of Pathology, and Bacteriology, John Radcliffe Hospital, Oxford*

SUMMARY In recent years cellular homologues of many viral oncogenes have been identified. As these genes are partially homologous to viral oncogenes and are activated in some tumour cell lines they are termed "proto-oncogenes". In tumour cell lines proto-oncogenes are activated by either quantitative or qualitative changes in gene structure: activation of these genes was originally thought to be a necessary primary event in carcinogenesis, but activated cellular oncogenes, unlike viral oncogenes, do not transform normal cells in culture. In experimental models cooperation between two oncogenes can induce transformation of early passage cells, and this has become the basis of an hypothesis for multistep carcinogenesis. Proto-oncogene products also show sequence homology to various components in the mitogenic pathway (growth factors, growth factor receptors, signal transducing proteins and nuclear proteins), and it has been postulated that they may cause deregulation of the various components of this pathway.

In human tumours single or multiple oncogene activation occurs. The pattern of oncogene activation in common solid malignancies is not consistent within any one class of tumour, nor is it uniform between classes, with three exceptions. In neuroblastoma, breast cancer, and perhaps in lung cancer there is relatively consistent activation of N-myc, neu, and c-myc/N-myc, respectively. Amplification of these genes generally correlates with poor prognosis. The introduction of methods for the direct study of oncogene transcription and their products will undoubtedly broaden our vision of cancer biology in man and, hopefully, add diagnostic and prognostic precision to tumour typing.

DNA transfection and other techniques have identified a class of dominant transforming genes from human and rodent tumour cells. Many of these transforming genes are the cellular homologues of acutely transforming viral oncogenes and are referred to as cellular oncogenes or proto-oncogenes. There are now more than 30 cellular oncogenes or proto-oncogenes. This class of gene has a physiological role in the regulation of cellular proliferation of differentiation. The designation proto-oncogene implies that these normal cellular genes need to be activated before they act in neoplasms. Their relevance as prime movers in the cancer process, however, is unconfirmed.

## Retroviral oncogenes and cellular oncogenes

Retroviruses containing oncogenes are the fastest acting carcinogens, and their oncogenes can initiate and maintain cancers. Temperature sensitive mutants of Harvey,[1] Kirsten,[2] and Fujinami[3] sarcoma viruses, and deletion mutants of avian erythoblastosis virus and other retroviruses,[4] have been genetically confirmed as being necessary for transformation. All susceptible cells infected by these retroviruses transform shortly after infection.

Retroviral and cellular oncogenes are structurally related. Almost all viral oncogenes are hybrids composed of coding regions from cellular oncogenes linked to the coding regions of essential viral genes. The first of the viral oncogenes shown to be a hybrid gene was the oncogene of an avian virus MC29.[5] It is the only gene encoded by MC29. About half of its information (1·5 Kb) codes for the gag gene of the virus. The other half, myc, is derived from the cellular oncogene c-myc. Cellular oncogenes are neither related, nor linked to retroviral sequences in normal cells.[5] On this basis only, therefore, cellular oncogenes differ considerably from their viral counterparts.

The single step oncogene hypothesis postulated that activation of an endogenous viral oncogene is sufficient to cause cancer[6] and that activation is the result of increased doses of the oncogene product. This view was corroborated by early experiments which suggested that the src gene of RSV, or the myc

gene of MC29, and the corresponding cellular oncogenes and their products were equivalent.[7] Cellular oncogenes can also be activated by mutations in the primary DNA sequence, such as c-Ha-ras.[8 9] Many cancers, however, are not caused by a single gene alternative but result from multiple events, probably involving multiple genes.[10] Retroviruses without transforming genes such as chronic leukaemia viruses and DNA viruses do not transform cells in culture and require long latent periods before the formation of neoplasms in vivo. Oncogenesis by these viruses thus seems to proceed by indirect mechanisms. This led to the multistep oncogene hypothesis, which postulates that an activated proto-oncogene is necessary, but not sufficient to cause cancer. A quantitatively or qualitatively activated proto-oncogene may function either as an initiation or as a maintenance gene, which acts together with another gene (viral or cellular) in a multistep process.[10 11]

Retroviral oncogenes and their cellular homologues may have a role in carcinogenesis in a multistep process. DNA from neoplasms induced by weakly transforming viruses can transform NIH 3T3 cells by DNA transfection. In initial experiments, however, no viral sequences could be detected in the transformed recipient cells,[12] suggesting that these cells contained transforming sequences unrelated to viral DNA. In T cell lymphomas induced by murine leukosis viruses it was postulated that cell proliferation was caused by binding of a virus specific product to mitogenic surface receptors.[13] Subsequently, it was proposed that integration of viral DNA in the vicinity of a potential cellular transforming gene resulted in abnormal gene expression through the action of the viral transcription promotor. The promotor insertion idea was supported by studies on B cell lymphoma induced by avian lymphoid leukosis virus (LLV).[14] In about 80% of these lymphomas viral DNA sequences, including the viral transcriptional promotor, are integrated in the vicinity of the cellular oncogene homologous to the transforming gene of the acutely transforming retrovirus MC29—that is, myc. Integration of viral DNA sequences apparently resulted in enhanced transcription of c-myc, implicating activation of this gene in lymphomagenesis induced by LLV.[14] Transfection of DNA from B cell lymphomas, induced by LLV, however, failed to show that the chicken myc sequence was integrated into NIH 3T3 cells transformed by lymphoma DNA.[15] Instead, another DNA sequence, B lym, was implicated,[16] indicating that such B cell lymphomas contained at least two activated oncogenes: (i) a c-myc gene activated by the integration of viral transcription regulatory sequences and; (ii) a distinct cellular gene (B lym) that is not linked to viral DNA and can efficiently induce the transformation of NIH 3T3 cells.

Activation of these two genes may correspond to events occurring at different stages of the neoplastic process. The earliest event detected in the course of LLV lymphomagenesis is the formation of multiple follicles within the bursa.[17] Most of the "preneoplastic" follicles regress, but a small fraction seem to progress to neoplastic cells. Thus viral activation of c-myc may be an early event that results in "preneoplastic" follicle proliferation. Progression to neoplasia might then entail further genetic changes resulting in the activation of another cellular oncogene (such as B lym). The identification of two oncogenes with a role in the development of LLV lymphoma is one example of progressive gene changes that occur during carcinogenesis in a variety of neoplasms.

## QUANTITATIVE MODEL OF ONCOGENE ACTION

It has been suggested that activation of c-myc is casually related to the development of human Burkitt's lymphomas, which are associated with Epstein-Barr virus infection. All Burkitt's lymphomas, with the exception of a single atypical Burkitt's lymphoma derived cell line,[18 19] carry one of three specific chromosomal translocations: c-myc is translocated to immunoglobulin (Ig) loci of chromosome 14, and less commonly, to Ig loci of chromosome 2 or 22. In the common translocations c-myc breaks at its noncoding 5′ end, or at variable distances upstream. The coding exons of the gene are transposed to the chromosome containing the immunoglobulin heavy chain gene, head to head with the immunoglobulin gene. In contrast, the variant translocations (those entailing the immunoglobulin light chain genes) break chromosome 8 below the tail end of the myc gene.[20] In these cases c-myc remains in its original location, and the constant region of the $\lambda$ or the $\kappa$ light chain became attached to it, in a head to tail orientation. Despite the considerable variability of the translocation breakpoint in and around the gene, the second and third exons of c-myc remain intact. The variation in translocation breakpoints within the immunoglobulin and c-myc loci also implies that no single nearby enhancer or promotor is responsible for myc activation in all tumours.

How does the c-myc/immunoglobulin juxtaposition contribute to the tumourigenic process? The mechanisms suggested have been abnormally high expression, abnormal transcript size, changed promotor use, mutations[21] and changed translational control. The crucial event may be more subtle than a relatively crude quantitative or qualitative change in that the myc gene juxtaposed with Ig becomes subject to cic control by the constitutively active immu-

noglobulin region and therefore behaves as if it were part of the Ig locus itself. The mechanistic role of these translocations in Burkitt's lymphomas has been adduced from work on cell lines. It could equally well be argued that the Burkitt's lymphoma cell line translocations have been selected by culture conditions because they confer a cell survival advantage in vitro, and do not reflect the events in Burkitt's lymphomas in vivo.

## QUALITATIVE MODEL OF ONCOGENE ACTION

The transformation of NIH 3T3 cells induced by DNA transfection from a human bladder carcinoma line (EJ/T24) led to the discovery of a DNA sequence homologous to the ras gene of Harvey murine sarcoma virus (Ha-MuSV).[8 9] Based on the viral model, the cellular Ha-ras oncogene (c-Ha-ras) was thought to be a potential cancer gene because it encodes a 21 000 dalton protein, $p21^{ras}$, which is colinear with the oncogene product p21 of Ha-MuSV. The c-Ha-ras from the bladder carcinoma cell line differs from its normal cellular counterpart in a point mutation in exon 1, which changes the 12th amino acid of $p21^{ras}$ from glycine to valine.[9] This mutation does not cause overproduction of the ras gene product (p21), nor does it change its affinity for GTP/GDP binding or the cellular location of ras protein.[22] The intrinsic hydrolysing activity of mutated ras protein, however, is about 10-fold lower than that of normal p21.[23] This change was thought to activate this gene to the functional equivalent of Ha-MuSV.

c-Ha-ras mutated at codon 12, has also been found in a high proportion of mammary carcinomas in rats induced by nitrosomethylurea.[24] Mouse skin tumours, including premalignant papillomas induced by chemicals, also showed mutations at codon 61 of c-Ha-ras. The prevalence of this mutation depended on the initiation agent used, but not the promotor, and the mutation was heterozygous in most papillomas but homozygous or amplified in some carcinomas.[25] These results suggested that mutation of c-Ha-ras occurs at the step of initiation, and further chromosomal changes at this locus may occur during tumour progression.[25]

Other members of the ras gene family also transform NIH 3T3 cells. c-Ki-ras, the cellular homologue of the ras gene of Kirsten sarcoma virus and N-ras, which is related to both Harvey and Kirsten sarcoma viruses, transform NIH 3T3 cells. Both c-Ki-ras and N-ras encode a p21 protein that is related to the product of c-Ha-ras. Mutation at codon 12 of c-Ki-ras is relatively common,[26] while N-ras is usually mutated at codon 61. These data, therefore, suggest that different ras proteins may have similar functions in regulatory pathways and that they share the same mechanisms of activation. Mutated ras genes, however, are unknown in biopsy samples of human tumours.

## OTHER MECHANISMS OF ONCOGENE ACTIVATION

Among the cellular homologues of retroviral oncogenes, c-myc and c-ras have been most intensively studied because they are commonly found in human tumour cell lines. Other cellular oncogenes whose biological properties are less well defined have also been found in cell lines. A human transforming gene homologous to chicken B lym-1 was identified in six Burkitt's lymphoma cell lines by DNA transfection. These genes are activated in B cell lymphomas of chickens and man.[16] The high incidence and high degree of species conservation observed in B lym-1 in these lymphomas suggest that it may regulate cell proliferation or differentiation. Human B lym-1 is not homologous to any known retroviral oncogene.

Human chronic myelogenous leukemia (CML) is characterised by a reciprocal translocation between chromosomes 9 and 22, resulting in an abbreviated form of chromosome 22 and the transfer of the cellular abl oncogene (a cellular homologue of Abelson murine leukemia viral oncogene) from chromosome 9 into the bcr (break cluster region) of chromosome 22. The resulting 8 Kb mRNA is a fused transcript of c-abl and bcr genes in which about 5·7 Kb is derived from c-abl and the rest from bcr genes.[27] The protein product of the transcript, like the normal c-abl homologue, has tyrosine kinase activity. The substitution of the amino terminus may change the conformation of the enzyme to trigger (perhaps constitutively) phosphorylation activity or the intracellular location of the enzyme. CML is the first example of a human cancer where a chromosomal translocation results in the production of a modified oncogene encoded protein which probably has a direct role in the malignant process.[27]

Amplification is another mechanism of oncogene activation. Amplification of c-myb ($\times 10$) and increased expression were observed in two cell lines derived independently from a single human colon adenocarcinoma. In contrast, c-myb expression was not detected in other solid tumours, including other colon carcinomas.[28] It was postulated that amplification and expression of c-myb may have contributed to the genesis of the tumour from which the cell lines were derived.

A cDNA clone of the c-sis oncogene derived from a cutaneous T cell lymphoma transforms NIH 3T3 cells. This implies that the c-sis transcript in the donor cell line contained the sequences necessary for transformation,[29] and that the protein of the c-sis gene, platelet-derived growth factor (PGDF), participates in the process. Addition of PGDF to cultured cells, however, and transfection of genomic DNA from this

lymphoma, were not capable of transforming NIH 3T3 cells, suggesting that other unidentified "cooperating" genes are essential for transformation.

Other transforming sequences have been identified by DNA transfection: these include mel (from a melanoma cell line)[30]; dbl (from diffuse B cell lymphoma)[31]; met (osteosarcoma cells)[32]; and trk (colon cancer).[33] In addition, the human cellular homologues of other viral or animal oncogenes including neu,[34] fes/fps,[35] mos,[36] and fms[37] have also been identified.

### COOPERATIVE ACTIVITY OF ONCOGENES

Activation of an oncogene may be only one step in the multistep process of carcinogenesis. When c-Ha-ras is introduced into early passage rat embryo fibroblasts (REF), transformations do not occur.[10] This is not due to an inability of the transfected gene to establish itself within REF; rather, REF do not respond to the encoded gene product. When transfected REF are dispersed and suspended in soft agar, colonies of transformants grow out indicating that one in vitro phenotype of transformation (anchorage independence) can be produced by mutated ras. This is a direct proof of the limited power of single oncogenes to transform primary cells and refutes the idea that a point mutation in c-Ha-ras is sufficient for carcinogenic development.

When cells are immortalised in culture—for example, NIH 3T3 cells—a subsequently introduced activated c-Ha-ras oncogene pushes the cells into a fully transformed tumourigenic state in a single step. Established cells thus seemed to possess all of the traits required for tumourigenicity save those that the activated oncogene specifies.[10] The ability of mutated c-Ha-ras to transform rat fibroblasts in culture depends on how often the cell line has been passaged. Cells that have been in culture of 10 passages are resistant to ras transformation while the same cells, maintained for 60 passages, transform easily.[38]

The changes that occur when a cell line becomes established in culture can be mimicked by genes of DNA viruses. In the case of polyoma virus three separate proteins (small, middle, and large T antigens) are coded by the "early" replication region of the genome that is active in polyoma transformed cells. The middle T antigen induces morphological change and anchorage independence, while large T antigen changes serum dependence and life span in culture.[39] The initial traits of transformation can be assigned, therefore, to distinct viral genes. This raised the question whether the phenotypes of establishment and immortalisation, which rendered cells reactive to the ras oncogene, could also be elicited by one or other of these viral oncogenes. When middle T and activated ras genes were cotransfected into REF, no new phenotypes were observed beyond those induced by ras alone, but cotransfection with large T and ras genes induced transformants which produced rapidly growing tumours in nude mice. The two oncogenes combined achieved complete conversion to tumourigenicity. Mutated ras can also cooperate with the early gene of adenovirus (Ela) to induce transformant foci.[40] The conversion of a normal cell into a tumour cell can thus be achieved by the cooperation of two distinct oncogenes, one cellular and one viral.

In some cases of B cell lymphomas and American Burkitt's lymphoma an apparently activated myc gene has been found, together with oncogenes such as B lym[16] and N-ras.[26] Perhaps the coexistence of these active oncogenes within these tumours reflects essential roles that they have together during tumourigenesis. Indeed, mutated c-Ha-ras and activated myc gene, when cotransfected to REF cultures, produce dense foci of transformants: acting together, c-myc and c-Ha-ras were able to do what neither could do separately. Similarly, N-myc, when linked to the long terminal repeats (LTR) of Moloney murine leukaemia virus, also cooperates with activated c-Ha-ras to transform primary cultures of REF, which can then form tumours in nude mice.[41]

These results partially explain why multiple cellular oncogenes are found in certain tumours. Each may perform a distinct function in tumourigenesis.

## Biological activity of cellular oncogenes

Based on biological activity, oncogene products are classified into several groups that are analogous to components of the mitogenic pathway, such as growth factors, membrane receptors, signal transducing proteins and nuclear proteins. Deregulation results in stimulation of growth or phenotypic change, or both.

Protein products of some oncogenes stimulate secretion of growth factors. Cells transfected by some oncogenes (such as ras, src, middle T, mos, fes, abl, fps, erb B, yes and mil/raf) release growth stimulating factors.[42] The growth factors are not encoded by the oncogenes themselves but by quite separate genes whose expression is indirectly stimulated by transfected oncogene. Some oncogenes, such as c-sis,[43] encode growth stimulatory proteins, and if deregulated, may assume the status of active oncogenes. Irrespective of whether increased secretion of growth factors is due to stimulation by oncogene products or by their direct transcription, cells must display the corresponding receptors on the cell membrane before a closed, positive feedback loop can be established. The establishment of these loops provides cells with a steady stream of growth stimulatory signals and frees them from dependence of growth factors imported from elsewhere.

Deregulation of the receptors of growth factors can also activate the mitogenic pathway. Here, the receptors themselves are changed in a way which continuously bombards the cell with growth stimulatory signals, even in the absence of growth factors. In this way the growth factor receptor assumes the role of an oncogenic protein. Three examples of this type have been reported. The first came from the work on the epidermal growth factor (EGF) receptor.[44] Sequencing of a portion of this receptor showed near identity with the protein specified by the erb B oncogene from the avian erythroblastosis virus.

The mitogenic pathway can also be activated by deregulation of proteins within the cells that transduce signals from growth factor receptors to targets further downstream. Ras proteins are good candidates as transducers of signals from cell surface receptors to intracellular targets because of their intrinsic GTP/GDP binding and hydrolysing activity. The GTPase activity of mutated c-Ha-ras is about 10 times lower than that of its normal counterpart.[23] When GTP binds to ras protein, it is activated to an excited state and sends out stimulatory signals to targets downstream. Stimulation stops on hydrolysis of GTP to GDP. Decreased GTPase activity of mutated ras protein may prolong its half life in the excited state, or the steady state concentration of $p21^{ras}$-GTP complex, or both, resulting in continuous signals. In this way the mitogenic pathway can be activated continuously, even when the stimulation of surface receptors is terminated. The EGF receptor also stimulates nucleotide binding by $p21^{ras}$. Ras protein stimulates the growth promoting effect of a variety of growth factors by stimulating inositol phospholipid metabolism, which participates in the signal transducing pathway. The growth promoting effect of EGF, however, which is independent of inositol phospholipid turnover, is also stimulated by ras proteins.[45] The exact mechanism of growth stimulation by mutated ras protein is still obscure.

Some oncogene proteins are located in the nucleus and these may have a role in growth control. The Ela oncogene of human adenovirus is a transacting regulator of transcription of other viral and cellular genes.[46] Cells transfected with myc oncogenes have an increased ability to promote expression of resident cellular genes as well as introduced genes, such as heat shock protein genes.[47] Myc protein may perturb the activity or specificity of the cellular transcription apparatus and mobilise the expression of a bank of cellular genes whose protein products are critical for growth and differentiation.[47] The normal cell genome carries multiple oncogenes whose products are nuclear (c-myc, N-myc, myb, fos, p53 and ski). Each of the proteins encoded by these genes may activate a slightly different group of cellular genes, but the abilities of most of them to affect transcription have yet to be shown. Growth factors also stimulate expression of myc, fos, and p53.

## Cellular oncogenes in human neoplasms

The role of oncogenes in real human cancers has been examined by three quite different methods applied to experimental systems: transfection assays; DNA, mRNA blots, and in situ hybridisation; immunohistochemical demonstration of oncogene products. Some of the data relating to cellular oncogene activation in human neoplasms are summarised below.

### BREAST

c-Ha-ras has been implicated in human breast cancer by DNA transfection. Of 21 human mammary tumours and cell lines,[48] only DNA from one carcinosarcoma cell line contained a transforming sequence, identified as mutated c-Ha-ras; every clonally derived cell line from this carcinosarcoma contained mutated c-Ha-ras. Cell lines derived from normal breast tissue of the same patient lacked transforming activity. In biopsy specimens c-Ha-ras mRNA was detected in only one of 23 cases of human breast cancer.[49] In our series (VTW Chan, JO'D McGee, unpublished observations) c-Ha-ras mRNA was detected by Northern blotting in 40% of breast cancers and in 25% of benign breast lesions biopsied. The c-Ha-ras gene was not mutated at codon 12 or 61 in any of these breast lesions. The different findings in these three studies indicate biological variation within breast cancers. Nevertheless, it is clear that mutation of c-Ha-ras is uncommon in breast cancer and that the expression of this gene is not systematic.

c-Ha-ras mRNA is present not only in malignant and benign epithelium but also in the stromal supporting cells (fibroblasts, endothelial cells, and smooth muscle cells of blood vessels) in mammary disease (fig 1–3). Immunohistochemically, ras p21 has been shown in normal mammary acini but it is not yet clear whether this is a c-Ha, N, or Ki-ras product.[50] In experimental mouse mammary tumours c-Ha-ras is also expressed by the same cell types but the stromal and epithelial cells of the overlying skin do not express it (fig 3). Although it is clear that c-Ha-ras expression is not an exclusive property of cancer cells in vivo, the presence of c-Ha-ras mRNA in stromal supporting cells of breast tumours raises intriguing questions. Additionally, these data (figs 1–3) underline the importance of combined molecular and cellular localisation data. If the nucelic acid extraction data (Chan VTW, McGee JO'D, unpublished observations) in this study were looked at in isolation, the erroneous assumption could be made that oncogene expression is a result of epithelial cell activity only.

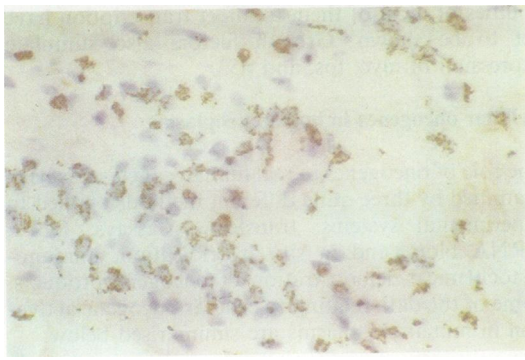


Fig 1 *c-Ha-ras mRNA in human invasive ductal cancer of breast. Carnoy fixed, frozen sections of breast were probed in situ with a biotinylated c-Ha-ras probe and site of hybridisation identified.*[65] *Many malignant cells show silver grains but some cells do not contain a signal. In sections pretreated with RNase A and T before in situ hybridisation, the signal largely disappeared, indicating that hybridisation (in fig 1) was due to c-Ha-ras mRNA.*

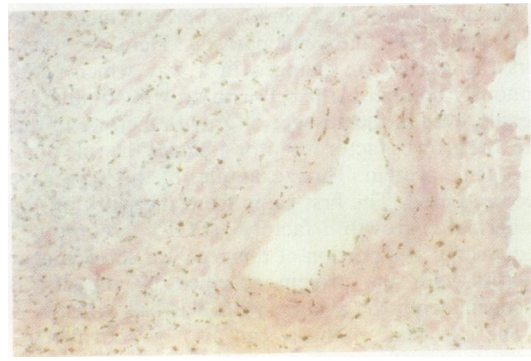
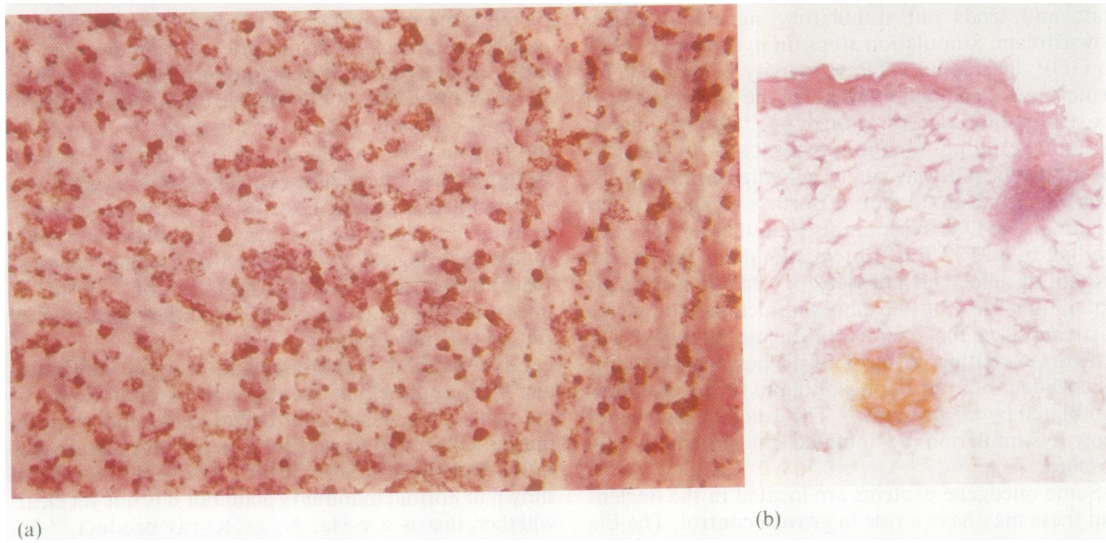


Fig 2 *c-Ha-ras mRNA in stromal cells of invasive ductal cancer of breast. Endothelial and smooth muscle cells of blood vessel, fibroblasts, and tumour cells contain c-Ha-ras mRNA. Detection as in fig 1.*




Figs 3a and b *c-Ha-ras expression in a mouse mammary tumour (a) and absence of expression (b) in overlying skin. mRNA was detected by in situ hybridisation as outlined in legend to fig 1.*

This point has general relevance to all studies in oncogenes.

Amplification of c-myc and non-germ line c-myc-related fragments occur in some breast cancer biopsy specimens and c-myc transcripts were raised in 10 of 14 cases. Of these 10 tumours, the c-myc gene was amplified in six cases. There was no correlation between genetic change of the c-myc domain and the level of mRNA.[50] In a separate study 87% of cancers and 43% of benign lesions had raised c-myc mRNA.[49] In the latter study N-ras (73%) and Ki-ras (65%) were also expressed in breast cancer and in about 30% of benign lesions. Expression of these cellular oncogenes, therefore, like c-Ha-ras, is not an exclusive property of malignant tumours.

A rare restriction fragment length polymorphism (RFLP) of c-mos has been found often in patients with breast cancers, and it was postulated that these patients have a particular susceptibility to breast cancer.[51]

Krontiris *et al* reported that the percentage of rare allelic lengths of c-Ha-ras is higher in patients with various tumours than that in controls, and they concluded that unusual alleles may be associated with susceptibility to cancer.[52] We have found no unusual alleles in breast cancers (Chan VWT, McGee JO'D, unpublished observations).

Recently amplification of the cellular proto-oncogene neu has been correlated with a bad prognosis, when prognosis is measured with time to relapse and overall survival.[53] It has been claimed that amplification of neu is more important than tumour size, patient age, and receptor status, and is independent of axillary node metastasis. If these observations are confirmed they will have considerable clinical relevance for biopsy diagnosis.

### LUNG

c-myc, N-myc, and c-Ki-ras are amplified in some human lung cancers. In 41 patients[54] c-myc and N-myc were at high (> 3 copies/haploid genome) or medium levels (1·5–3 copies/haploid genome). A quite separate myc gene (L-myc) is amplified in small cell carcinomas of lung (SCCL), but this occurs in only about 10% of samples analysed.[55] This gene was also expressed in these lung tumours and cell lines with amplified L-myc, but one cell line without an amplified gene also showed L-myc transcripts. c-myc amplification was initially thought to be associated with an aggressive histopathological tumour type[16] but this is in doubt.[54]

### COLON

In colonic cancers activated c-Ki-ras and N-ras were found in four of 28 cases.[33] c-Ha-ras has also been implicated in colonic cancers. Nine of 17 primary colonic cancers had substantially raised concentrations of ras protein compared with adjacent normal tissue. There was no correlation between ras p21 and tumour stage or metastases; in fact, the reverse was found. Eight of the nine ras positive tumours were Dukes' B or C, while Dukes' "D" (four of five cases) had normal concentrations. In metastatic deposits all nine cases showed considerably reduced concentrations of p21. These findings were interpreted as indicating that p21 plays a part in the early stages of colonic cancer but that it is not essential for tumour progression and spread.[56] This is supported by the observation that both c-Ha and c-Ki-ras are expressed at high concentrations in premalignant colonic polyps.[57]

### LYMPHOMA AND LEUKAEMIA

c-abl expression is typical of CLL. The novel abl transcript in CLL is induced by a t(9–22) translocation[27] and seems to be specific for this leukaemia as it has only been found in one of 25 other leukaemic patients.[26] This is strong evidence that these abl transcripts have a role in the genesis of CLL.

In a relatively comprehensive analysis of oncogene expression in acute lymphocytic leukaemia c-myc and c-myb were detected in all cases at variable concentrations. No correlation, however, was observed between rates of transcription and cell proliferation, or stage of differentiation. Conversely, significant amounts of c-fos transcription were detected only in myelomonocytic and monocytic leukaemia; c-Ha-ras was uniformally expressed at low levels in neoplastic and non-neoplastic white blood cells; c-Ki-ras expression was found only in T ALL, while N-ras transcription was barely detected.[58]

In addition to c-myc activation and translocation in Burkitt's lymphoma, this gene is also translocated (t8–14) in some cases of acute lymphocytic leukaemia (ALL). In ALL, high concentrations of two different c-myc transcripts were detected and these differed in size from normal c-myc mRNA. In transfection assays activated N-ras, mutated at codon 13, has also been described in acute myelogenous leukaemia.

### TUMOURS OF OTHER SYSTEMS

In spite of the fact that mutated c-Ha-ras was identified in a bladder cancer cell line (EJ/T24) only two of 23 urinary tract tumours contained transforming DNA. In one case ras was mutated at codon 61, while in the other neither codon 12 nor 61 were mutated.

Striking amplification of the N-myc gene occurs in neuroblastoma. In 12 of 63 cases the amplification was 100 to 300-fold and three to 10-fold in a further 10 cases. Amplification is highly correlated with disease stage—that is, all 24 cases with N-myc amplification were stage 3–4.[59] In retinoblastomas N-myc is expressed at high concentrations, but this does not invariably correlate with N-myc amplification.[60]

Other cellular oncogene anomalies have been recorded in a variety of tumours, such as c-erb-2 in salivary tumours, c-raf in gastric cancers, and mutated c-Ki-ras in ovarian cancer.

Amplification or deletion, or both, of oncogenes occur in some human tumours.[61] In 101 tumours from different sites no detectable amplification of c-Ha-ras was observed, but there was apparent loss of one c-Ha-ras allele in some. On average, about 18% showed allelic deletion, and this was twice as common in metastases (29%) as in primary tumours (15%). Deletion of c-myb was also observed in some tumours. The average percentage of tumours having c-myb deletion was 11% but there was no difference between primary and metastatic tumours. In contrast, amplification of c-myc was observed in 10% of these

tumours. Amplification was higher in metastatic tumours (5–8 times) than in primary tumours (3–5 times), suggesting a correlation between c-myc amplification and tumour metastasis or progression. Interestingly, amplification of c-myc was not seen in haematological malignancies in which c-myc was thought to be active in tumourigenesis. This agrees with Rothberg's data, in which only one case of Burkitt's lymphoma among 106 cases of fresh leukaemias and lymphomas showed amplification of c-myc.[62]

Multiple oncogene expression has also been shown in many tumours,[63] but oncogene transcription, although higher than in the corresponding normal tissue, is not an exclusive property of cancers. This is not unexpected as several oncongenic proteins have been shown in normal tissue immunohistochemically. Ras proteins are present not only in proliferating cells but also in terminally or highly differentiated cells such as neurones, ganglion cells, nerve, smooth muscle and pancreatic islets. In fact, the amount of ras and src transcripts and their respective proteins are 10 × higher in normal brain and heart than in other normal organs, and higher than in many tumours.[64]

Although oncogenes may be amplified (or deleted) and expressed in human cancers, it is clear that only a fraction show oncogene activation. Furthermore, there is no consistent pattern of oncogene activation in most cancers except some leukaemias, neuroblastomas, and perhaps breast cancer. This suggests that oncogene activation is not common in human cancers and when present, may be a result rather than a cause of tumourigenesis. The expression of some oncofetal proteins (which are casually irrelevant to the malignant growth process) is more consistent than oncogene expression in tumours.

In clinical practice oncogene amplification correlates with prognosis in neuroblastomas[59] and perhaps also in breast[54] and lung[55] cancer. Whether measurement of oncogene transcription and their products will prove a useful adjunct to histopathological staging of other tumours remains open. There is little doubt, however, that as in situ hybridisation becomes a routine procedure the transcription of many proto-oncogenes and the mutated genes themselves will be visualised in human tumour biopsy specimens. We suspect that the outcome may be similar to that of immunohistochemistry in histopathology. Our vision of cancer biology will broaden and become more precise. If there are unique genetic markers of cancers the proof of this will only come from showing their presence in real tumours and not in cells growing on plastic. It would be naive, however, to expect that there will be a universal genetic marker for all malignant cells when it is remembered that similar clinical haemoglobinophathies are caused by quite different defects in the globin genes.

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Requests for reprints to: Professor J O'D McGee, University of Oxford, Nuffield Department of Pathology, Level 1, John Radcliffe Hospital, Oxford OX3 9DU, England.