The Role of ras and myc Oncogenes in Human Solid Tumours and Their Relevance in Diagnosis and Prognosis (Review)

JOHN K. FIELD and DEMETRIOS A. SPANDIDOS

1 Department of Clinical Dental Sciences, School of Dentistry, University of Liverpool, Pembroke Place, PO Box 147, Liverpool L69 3BX, England;
2 Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, 48 Vas. Constantinou Ave., Athens, 11635;
3 Medical School, University of Crete, Heraklion 71409, Greece

Abstract. Advances in the field of oncogenes have produced a tool to investigate the different stages in multistep carcinogenesis. The role of the ras and myc gene families have been extensively investigated in the progression of carcinogenesis in a range of human solid tumours. This review critically analyses the data available on the role of these oncogenes in the six most common cancers worldwide, (i.e. cancer of the stomach, lung, breast, colon, cervix, and mouth and pharynx). In certain cases the incidence of aberrant gene expression and genetic alterations of the ras and myc gene families have been shown to be important in the progression of these cancers and may be of use as prognostic indicators.

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1. Introduction

The transformation of a normal cell into a cancer cell is a complex multistep process resulting in a clone of cells that are no longer under normal regulatory control. Recent advances in the field of oncogenes have provided a tool to investigate the different steps in carcinogenesis, both at an experimental level and also by analysing human tumours. If the control mechanisms of certain genes could be identified at particular steps in tumour development, then it might be possible to develop clinical strategies for intervention, to block further tumour progression.

The evidence that transforming genes were involved in tumourigenesis came initially from work with retroviruses, which could transform cells in culture and induce tumours in experimental animals (1). The realisation that the acutely transforming retroviruses had transduced their v-onc genes from the host cell led to the discovery of a number of proto-oncogenes in normal cells (2). Subsequently, it was discovered that these normal genes or proto-oncogenes were activated in certain tumours and appeared to be involved in the progression of the disease (3, 4, 5).

Proto-oncogenes may be activated by a number of methods; insertion of a promoter or enhancer element, translocations, point mutations of a structural gene and amplifications. These mechanisms may lead to an increase in the oncogenic product or may produce an altered gene product resulting in malignant transformation of the cell (4,
5). Other mechanisms as yet unidentified must exist to explain over-expression of certain oncogenes in tumors without obvious genetic alterations. These mechanisms may take the form of other cellular genes influencing the oncogene regulation, or the stability of the oncogene mRNA, of which little is known.

It is now clear that certain tumor suppressor genes or anti-oncogenes may also be involved in tumour progression (for review see ref 6). The existence of these genes has been inferred by both experimental fusion of normal and cancer cells which often suppresses the neoplastic phenotype (7) and by the study of certain forms of hereditary cancer (8, 9, 10). Recently an association between certain chromosomal deletions and cancer of a non-heritable origin has been reported (for review see ref. 11).

The aim of this review is to elucidate the role of the ras and myc gene families in the six most common solid tumors and also to discuss the relevance of these genes to the diagnosis and prognosis of these carcinomas. Deletions in chromosomes that are pertinent to the role of ras and myc genes will also be discussed.

2. Tumour development

Carcinogenesis is considered to be a multistep process. Evidence for this comes from epidemiological studies in man, biochemical and histopathological analysis of tumors in vitro and in vivo, cell tumor formation studies with chemicals, radiation and viruses (for review see refs 12, 13). Carcinogenesis may be divided into three distant stages: initiation, promotion and progression.

Initiation is possibly an absolute requirement involving mutational events which are therefore irreversible. Initiation may occur very rapidly with the use of a single application of a carcinogen. This has been observed in a number of animal model systems where certain chemical carcinogens acting with or without tumor promoters induce the activation of the ras genes by point mutation (14-18). It is feasible that the point mutations in the ras gene confer a selective growth advantage on these cells. It is not unreasonable to suggest that mutations in other genes probably also occur; some of these mutations will be lethal while others give the cells a competitive advantage and in time undergo phenotype diversification and eventually become a malignant subpopulation. There is also evidence for X-rays inducing point mutations in the ras proto-oncogene in lymphomas (19). Viral initiation has also been reported in a number of model systems (20).

Evidence from animal model systems has shown that with the use of a particular carcinogen, certain tumor types are formed which carry specific mutations in the ras gene family (17-19, 21, 22). A major breakthrough in the understanding of initiation came from the work by Balmain et al (14), which demonstrated that a high percentage of mouse skin papillomas had an activated H-ras oncogene. The fact that this gene was activated in the premalignant state of skin carcinogenesis supports the hypothesis that this point mutation was intimately linked with initiation. Experimental evidence suggests that over-expression of a single mutated ras oncogene is sufficient to transform primary rodent cells into a malignant phenotype (23). Also in early passage rodent cells transformed with recombinant plasmids the mutant T24 H-ras oncogene was shown to set into motion the malignant conversion, by causing multiple metastasis when intravenously or subcutaneously injected into nude mice (24). The myc gene has also been shown to act as an initiator of carcinogenesis when the gene is introduced into cells in a retrovirus or in transgenic mice (25). Even though the ras and myc genes have been implicated in the initial stages of tumorigenesis, it is most likely that there are more general effects, such as the ability of cells to incorporate and metabolise carcinogens, whether the affected cells are proliferating or non-proliferating, and what selection pressures exist within the tissues.

Tumour promotion is the second stage in the classical model of multistep carcinogenesis. However, the evidence for DNA damage is lacking at this step in carcinogenesis. The major advance in this area has been the identification of protein kinase C as a binding site for the tumor promoter TPA. (26, 27). The role of tumor promoters remains unclear (for a review see refs 28, 29). Hesnins and Yuspa (29) disagree with the two stage model for phorbol ester promotion. Several test systems have been investigated to ascertain the effects of first and second stage promoters, but as yet our understanding of tumour promoters is incomplete (20, 30, 31).

The progression of the benign tumor to the malignant phenotype is the most important clinical stage. However, at the early stages it is not possible to differentiate experimentally between promotion and progression of the disease, and little is known about the molecular changes involved. Possibly genetic alterations, such as chromosomal rearrangements, deletions, mutations or amplification of specific genes may be important at this stage. Genetic characteristics of malignant cells are aneuploidy, double-minute chromosomes and homogeneously staining regions in chromosomes (32, 33). Also the DNA content per cell is often increased in cancer cells when measured cytochemically (34, 35). Recently Vogelstein et al (36) have demonstrated that patients suffering from cancer of the colon with a higher ratio of allelic deletions have a poorer prognosis than those with a lower ratio.

There are now a large number of reports concerning oncogenes in human tumors, some of which have been identified at the different stages of carcinogenesis, on the basis of clinical and pathological evidence. Therefore it is clear that oncogenes can act at all three recognisable stages: initiation, promotion and progression. The question this review addresses is at what stages the ras and myc oncogenes are involved in the evolution of the common human carcinomas.
3. The role of ras and myc gene families in carcinogenesis

The role of the ras and myc gene families in the progression of carcinogenesis in solid tumours has been investigated in a wide range of tissues. The six most common malignancies worldwide are cancer of the stomach, lung, breast, colon, cervix and mouth and pharynx (37) (Figure 1). They have all been examined to a greater or lesser extent to ascertain whether the myc and ras oncogenes are important in their evolution. The incidence of aberrant gene expression or genetic alterations may also be of use as prognostic indicators.

However, even though a large number of studies have been undertaken, few have yielded any real advancement in our knowledge regarding the role these genes play in tumourigenesis. In an effort to dissect out which of these studies further our understanding on the role of oncogenes in carcinogenesis, certain clinical and statistical requirements have to be taken into consideration.

Initially, studies on oncogene expression and alteration of these genes were undertaken in a wide range of human tumours, but with few samples of each tissue type. Even though this provided evidence for the presence of oncogenes in tumours, little could be deduced about their role. In the past six years a large volume of work has been undertaken in this field and much of it falls into pilot or preliminary studies. In many instances there have been insufficient numbers of patients examined for reliable statistical analysis to be undertaken. Also in many cases there have not been complete clinical data with follow up. There are many good reasons for this happening, as publication would be delayed for 3-5 years after analysing fresh tumour tissue to await follow up data and produce survival curves.

In order to separate out the different stages of carcinogenesis it is of particular benefit to study those cancers that have defined clinical stages, as in benign, premalignant, primary tumours and subsequent metastasis. Taking these points into consideration, there are few studies that have all these clinical and statistical requirements.

It is extremely difficult to make any broad generalization about the role of ras or myc tumourigenesis, as such a range of results have been published; for instance c-myc overexpression in carcinoma of the uterine cervix correlates with risk of relapse (38); c-myc amplification correlates with a poor prognosis in breast cancer patients (39), elevated c-myc oncoprotein correlates with a poor prognosis in head and neck cancer (40) and N-myc amplifications are associated with rapid tumour progression in neuroblastomas (41). Whereas in colorectal carcinoma c-myc expressions did not correlate with patients survival (42) and N-myc and L-myc did not correlate with the clinical outcome of patients with small cell lung cancer (SCLC) (43).

Moreover, there is not always an agreement of results between different research groups using the same cancer type. This is very noticeable in breast cancer investigations, where no correlation was found between the 57 per cent incidence of c-myc amplification and prognosis (44) and in another study the incidence of c-myc amplifications was found to be very low (6 per cent) and again of no prognostic importance (45). Also different results have been found for N-myc expression in SCLC and c-myc in colon cancer when correlated with survival (53, 77).
The use of oligonucleotides (46) and RNase mismatch cleavage analysis (47) has changed scientific opinion on the incidence of ras mutations in human tumors. It is now realised that point mutations in the K-ras genes are much more common than originally thought and account for 40 percent in colon tumors. However, no correlation was found between the presence of the mutated ras oncogenes and the degree of invasiveness in colon cancer (46, 47), whereas mutation in the H-ras gene is significantly associated with poor prognosis in cervical cancer (48, 49). An extremely interesting observation emerged from the colon tumor investigations (46, 47), indicating that mutations in ras protooncogenes were detected in premalignant colon polyps. Ras mutations have also been found in human pre-leukemia myelodysplastic syndrome (50, 51). Even though amplification of the ras genes in tumors is a rare event, overexpression of RNA transcripts of these genes has been reported in the colon (52, 53) and overexpression has been considered important in the clinical course of the disease.

Clinically defined potentially malignant lesions are recognised in both breast and colon cancer, and this provides a very useful tool to investigate the switching on of certain oncogenes in the progression of the disease. It appears from the limited data available in the benign cystic disease of the breast that the c-myc gene is amplified in some cases (39, 44) and that c-myc expression was elevated (54).

The situation in colon polyps has been more fully investigated and there is a consensus of opinion in the literature that elevated ras expression is associated with this premalignant stage of colon cancer (55, 56).

The most reliable data that can be considered to be of prognostic importance are those which have been subjected to thorough statistical analysis such as disease-free survival rates computed by Kaplan-Meier (57), Cox's proportional hazard model (58) or the log rank test (59). These types of analysis have been carried out in breast cancer (39, 60, 61), colon cancer (42), small cell lung cancer patients (43), cancer of the uterine cervix (38), and in head and neck cancer (40). It is only with detailed attention to this type of statistical survival data that we may draw meaningful conclusions from these oncogene studies. As both ras and myc genes appear to have different roles in many human tumour types, each of the six most common solid human tumors will be discussed separately.

4. Stomach cancer

4i. C-myc expression in stomach carcinomas. There is little information on c-myc genetic alterations in stomach carcinomas, apart from the report by Yokota et al (62) who looked at nine stomach carcinomas in a survey of 71 epithelial cancers. No amplification of the c-myc gene was found in the stomach specimens, compared with a total of eleven per cent in other tumors studied.

The expression of the c-myc gene in gastric cancers has been investigated using the monoclonal antibody 1-9E10 (63). Low levels of expression of the protein product p62 c-myc were found in normal gastric tissue, compared to those found in inflammatory, metastatic and dysplastic specimens (64), suggesting that this oncogene product maybe of use in identifying potentially neoplastic hyperproliferative states. However, the results of Allum et al (65) are not in agreement with these findings. Allum et al (65) used myc 1-6E10 to detect c-myc p62 in 93 specimens of gastric cancer; they found that less than 40 per cent of the tumours contained positively staining cells, and also that there was no correlation with the degree of histological differentiation.

4ii. Ras genetic alteration and expression in stomach cancer. In the study of 71 epithelial tumors of which 9 were from the stomach, no H-ras deletions were reported by Yokota et al (62). However, with the use of oligonucleotide probes, Bos et al (65a) found a gastric carcinoma with a mutated K-ras allele, and also an amplified normal K-ras allele. They suggest that these two changes in the K-ras genes may indicate two separate steps in the genesis of this particular gastric carcinoma unless the tumor had two separate clonal origins.

The expression of the ras oncogene product p21 has been investigated using a number of monoclonal antibodies, HAS6, HAS5, HAS2, (66) RAP-5 (67, 68), RAS K1-16 (69) and also by a direct binding liquid competition radioimmunoassay (RIA) to the p21 protein product (70).

Using the RAP5 monoclonal antibody the p21 ras oncogene was detected in only 1 of 13 cases of normal or benign gastric lesions compared to all of the 20 gastric carcinomas tested (67). A similar result using the same monoclonal antibody, was found in 65 of 96 stomach cancers (68). Ras p21 was quantified by De Biasi et al in malignant and normal stomach cancer using a direct binding liquid competitions assay (RIA) (70); they reported that the amount of p21 ras expressed in malignant stomach cancer was significantly greater than that found in benign tissues (P<0.005). Yoshida et al (69) reported on the isolation of 16 murine monoclonal antibodies to ras p21, and they investigated the expression of p21 ras in 101 cases of stomach cancer and in 52 cases of non-stomach cancer. Their results indicate that p21 ras is expressed in moderately well differentiated stomach cancer, intestinal metaplasia and in atypical hyperplasia. In a recent study, 174 gastric cancers were investigated for the expression of TGFα and H-ras p21 immunohistochemically. TGFα immunoreactivity was detected in 7 of 27 early carcinomas and in 110 of 147 advanced cancer (P<0.01). Patients with carcinomas showing expression of both TGFα and H-ras p21 (59 of 67 cases) had a very poor prognosis compared to those with low levels of expression (P<0.05) (71).

The data available on c-myc and ras gene action in gastric carcinomas indicate that increased ras expression is important in the progression of stomach cancer, but the timing of c-myc is still uncertain.
5. Lung cancer

There are four major histological types of lung cancer, squamous cell carcinoma, adenocarcinoma, large cell carcinoma and small cell lung carcinoma (SCLC). The former three groups are called the non-small cell lung carcinomas (N-SCLC) and have different clinical features from the SCLC. The SCLC are also treated differently, usually with combination chemotherapy and radiotherapy and have a poor prognosis (72).

There have been a large number of reports on the molecular analysis of lung cancer (reviewed by Minna et al., 72, 73) but the majority of the investigations have concentrated on SCLC (43, 74-79). The interpretation of the results in SCLC is complicated by the fact that the majority are from SCLC cell lines or from necropsy specimens post-chemotherapy treatment; however, there are a number of examples taken from fresh primary SCLC prior to any chemotherapy and radiotherapy.

5i. Myc and ras expression and amplification in SCLC. A large number of SCLC cell lines have been set up and all members of the myc gene family have been investigated. In 31 SCC cell lines, 14 have had a c-myc or N-myc gene amplified with or without over-expression (75). It is of considerable interest that these authors also found N-myc amplification in a tumour cell line prior to chemotherapy and N-myc amplification in a tumour metastasis.

A survey of the expression and amplification of 16 proto-oncogenes in 12 SCLC cell lines showed that 7 out of 12 had c-myc amplification, 3 out of 12 had N-myc amplification and 1 out of 12 had simultaneous amplification of c-myc and N-myc. All the cell lines had similar levels of expression of N-ras, K-ras, H-ras and c-ras1 but no amplification of these genes (79). The other oncogenes studied showed no significant expression.

Amplification of the myc gene family has also been investigated in 44 SCLC cell lines, of which 19 were established before chemotherapy was initiated. C-myc amplification was only seen in cell lines established from treated patients. It was demonstrated that the chemotherapy treated cell lines with c-myc amplification survived a significantly shorter time than patients without c-myc amplification (P<0.05) (78).

In another investigation (43) into 38 different SCLC specimens (34 from necropsy, 4 from surgery prior to treatment), it was found that 4 out of 38 had N-myc amplification and 2 out of 38 had L-myc amplification but none had c-myc gene amplification. All 6 tumour specimens with myc amplification were taken from patients who had been treated with combination chemotherapy. However, no difference in the clinical course of the disease was found between patients with and those without N-myc or L-myc amplification. A particular problem with analysing survival data for SCLC is that these patients have such a poor prognosis anyhow (usually less than 12 months), that one has to be cautious in interpreting the data.

In another study 15 primary biopsies from patients who had SCLC but had no previous treatment, were investigated for N-myc expression using in situ hybridisation techniques (77). The results indicated that increased N-myc expression correlated with poor response to chemotherapy, rapid tumour growth and short survival (P<0.01). However, the most likely explanation for the patients without N-myc expression having a longer survival was that they had extended treatment periods of cytostatic drugs compared to the patients with higher levels of N-myc expression. Even so, this study provides very valuable information on fresh primary untreated SCLC specimens, as it indicates that N-myc is important in the progression of the disease. The results in fresh tumour specimens indicate that the N-myc gene is over-expressed in 6 out of 15 cases of SCLC before chemotherapy (77) and amplified in 4 out of 4 cases after the combined chemotherapy (43). The mechanism for overexpression of N-myc in the 6 specimens may not be due to amplification but it is clear that the N-myc gene is unregulated in SCLC regardless of whether the tumour has received chemotherapy. This provides further evidence for the involvement of N-myc in SCLC and that it is not just an indication of genetic damage due to cytostatic drugs. Recently it has been demonstrated that c-myc is overexpressed in a number of biopsy specimens taken from untreated bronchial carcinomas using the myc 1-9E10 monoclonal antibody (135). Elevated c-myc expression was found in 16 of 37 (43%) squamous carcinomas; 4 of 10 (29%) adenocarcinomas; 3 of 7 (42%) N-SCLC's and 4 of 21 (19%) SCLC's. However no correlation was found between elevated c-myc expression and survival of these patients (135).

In contrast to these results in SCLC, there has been a report of K-ras and c-myc amplification with a point mutation in K-ras in a lung giant cell carcinoma (LGCC) (80). This gives weight to the theory of oncogene cooperation in carcinogenesis, but the stage at which these two oncogenes were activated is unknown.

In the context of timing of the activation of oncogenes in lung cancer, the results of Rodenhuis et al (81) contribute to this. They examined ras gene mutations in N-SCLC patients, the majority of specimens being obtained at thoracotomy, and they found that 9 out of 35 adenocarcinomas of the lung had K-ras mutations. On examining the patients' smoking history, they concluded that there was an association between their smoking habits and the incidence of K-ras mutations in their lung cancers. It is possible that K-ras mutation events may be directly related to carcinogenic substances in tobacco smoke and therefore that these mutations are occurring at the initiation stages of these cancers.

The differential expression of ras p21 in 23 fresh primary lung tumours (82) had been correlated with histological classification; 9 out of 11 tumours with a squamous histology compared to 1 out of 12 non-squamous carcinomas of the
Table I. Review of ras and myc expression and genetic alterations in breast cancer.

This table has been compiled from the published data pertaining to the role of ras and myc gene families in breast cancer. In order to make comparisons between the data from different papers, the original authors’ results have been expressed in a different format in a number of cases. The results have been broken down into: malignant, potentially malignant and benign lesions with the number of patients in each group. Patients with amplification and elevated expression are shown as percentages. Data on rearrangements and mutations are described in the clinical correlation section.

Any clinical correlations pertaining to the review are also included.

Table 1A.

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Tumour type</th>
<th>No. of patients studied</th>
<th>Percentage of patients with amplification</th>
<th>Percentage of patients with elevated expression</th>
<th>Clinical correlations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-myc (RNA)</td>
<td>Carcinoma</td>
<td>23</td>
<td></td>
<td>73</td>
<td>Significant difference in ras and myc expression between benign and malignant tumour (P&gt;0.01)</td>
<td>105</td>
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<tr>
<td></td>
<td>Fibrocystic disease</td>
<td>23</td>
<td>17</td>
<td>0</td>
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<td></td>
<td>Fibroadenoma</td>
<td>4</td>
<td></td>
<td>0</td>
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<td></td>
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<tr>
<td>c-myc (mRNA)</td>
<td>Carcinoma</td>
<td>121</td>
<td>32</td>
<td>70</td>
<td>c-myc amplification correlates with pt age P&lt;0.002 and invasive ductal histology. 6 of the 14 carcinomas with elevated c-myc expression had a amplified c-myc gene</td>
<td>87</td>
</tr>
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<td></td>
<td>Fibroadenoma</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carcinoma</td>
<td>14</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>c-myc</td>
<td>Carcinoma</td>
<td>41</td>
<td>17</td>
<td>24</td>
<td>c-myc amplification correlated with poor prognosis P&lt;0.02. (Number of pts analysed for c-myc expression unclear from original paper)</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Lymph node metastasis</td>
<td>10</td>
<td>40</td>
<td></td>
<td></td>
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<tr>
<td>L-myc</td>
<td>Carcinoma</td>
<td>41</td>
<td>24</td>
<td></td>
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<td>N-myc</td>
<td></td>
<td>41</td>
<td>0</td>
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<tr>
<td>c-myc (RNA)</td>
<td>Fibrocystic disease</td>
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<td>10</td>
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<tr>
<td>c-myc</td>
<td>Fibroadenoma</td>
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<td>0</td>
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<td>c-myc</td>
<td>Fibrosarcoma</td>
<td>1</td>
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<td>c-myc (RNA)</td>
<td>Carcinoma</td>
<td>100</td>
<td>6</td>
<td>45</td>
<td>High levels of c-myc expression correlated with lymph node metastasis (P&gt;0.01)</td>
<td>45</td>
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<tr>
<td></td>
<td>Carcinoma</td>
<td>98</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td>Fibroadenoma</td>
<td>6</td>
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<td>0</td>
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<tr>
<td>c-myc (southern)</td>
<td>Carcinoma</td>
<td>37</td>
<td>57</td>
<td></td>
<td>No clinical correlations</td>
<td>44</td>
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<tr>
<td></td>
<td>Fibroadenoma</td>
<td>7</td>
<td>29</td>
<td></td>
<td>Rearrangements with amplification seen in 3 malignant tumours</td>
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<tr>
<td></td>
<td>Cystosarcoma</td>
<td>2</td>
<td>50</td>
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</tbody>
</table>

continued
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Table IA continued

<table>
<thead>
<tr>
<th>Onco gene</th>
<th>Tissue type</th>
<th>No. of pts.</th>
<th>Percentage with elevated expression</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-myc (myc 1-9E10)</td>
<td>Fibroscatic disease</td>
<td>198</td>
<td>0</td>
<td>c-myc protein found at a higher level in mucous metaplastic cells and multiple papillomas</td>
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<tr>
<td>C-myc (ELISA)</td>
<td>Carcinoma</td>
<td>24</td>
<td>100</td>
<td>Weak correlation with patients age P&lt;0.1 No correlation with prognosis</td>
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Table IB.

<table>
<thead>
<tr>
<th>Onco gene</th>
<th>Tissue type</th>
<th>No. of pts.</th>
<th>Percentage with elevated expression</th>
<th>Clinical correlations</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>H-, K-, N-ras</td>
<td>Carcinoma</td>
<td>104</td>
<td></td>
<td>No rearrangements or amplification found, one allele lost in 14 of 51 patients heterozygous for H-ras, correlated with grade III tumours.</td>
<td></td>
</tr>
<tr>
<td>K- or N-ras</td>
<td>Carcinoma</td>
<td>22</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-ras</td>
<td>Carcinoma</td>
<td>22</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ki-ras</td>
<td>Carcinoma</td>
<td>22</td>
<td>0</td>
<td>Breast cancer cells express H-ras p21 but not N-ras p21 or K-ras p21</td>
<td></td>
</tr>
<tr>
<td>N-ras (mRNA) (Southern)</td>
<td></td>
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<tr>
<td>H-ras (oligonucleotides)</td>
<td>Carcinoma</td>
<td>24</td>
<td>100</td>
<td>2-G-T mutations (codon 12) amplified 2 loss of H-ras allele.</td>
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<td>H-ras (RNA)</td>
<td>Carcinoma</td>
<td>12</td>
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<td>H-ras (RNA)</td>
<td>Carcinoma</td>
<td>24</td>
<td>100</td>
<td>Elevated expression correlated with histological grade.</td>
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<td>N-ras</td>
<td>Carcinoma</td>
<td>23</td>
<td>65</td>
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<td>K-ras</td>
<td>Carcinoma</td>
<td>48</td>
<td>4</td>
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<tr>
<td>H-ras</td>
<td>Fibroadenoma</td>
<td>26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-ras (RNA)</td>
<td>Fibrocystic (27)</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ras (RNA)</td>
<td>Fibroadenoma disease</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-ras</td>
<td>Carcinoma</td>
<td>41</td>
<td></td>
<td>No amplification observed</td>
<td></td>
</tr>
<tr>
<td>ras p21</td>
<td>Carcinoma</td>
<td>30</td>
<td>63</td>
<td>Correlation between p21 ras and tumour invasion, (positive staining &gt;20% of tumour carcinoma cells scoring positive)</td>
<td></td>
</tr>
<tr>
<td>RAP-5 (mAb)</td>
<td>Fibroscatic</td>
<td>11</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ras p21</td>
<td>Fibroadenoma</td>
<td>10</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ras p21 (mAb)</td>
<td>Carcinoma in situ CA.</td>
<td>18</td>
<td>83</td>
<td>No clinical correlations found</td>
<td></td>
</tr>
<tr>
<td>Y13-259 (mAb)</td>
<td>2</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

continued
lung demonstrated increased ras p21 expression. Baylin (83) has suggested that there is a developmental relationship between the histological subtypes of the main lung cancers with some tumours showing features of more than one subtype. Kuzrock et al. (82) argues that this may explain why one adenocarcinoma had elevated ras p21 and that the over-expression of the ras gene is involved in the evolution of the squamous cell carcinomas.

Recently another variable has been uncovered in the genesis of lung cancer, with the demonstration of a deletion in the chromosomal region 3p21 in all major types of lung cancer, i.e. both N-SCLC and SCLC (84). These authors concluded that loss or inactivation of a gene on 3p21 was involved in the development of all lung cancers. This view is contrary to that reported by Brauch et al. (85), who found loss of alleles at 3p a consistent feature in SCLC and only occasionally in N-SCLC. Based on the results in Johnson’s paper (78), Brauch et al. (85) calculate that myc gene amplification is absent from 89 percent of cell lines from untreated patients. Using these figures they argue that, as myc amplification is absent in untreated SCLC patients but as 3p deletions are deleted in cell lines from untreated patients, then in terms of multistep carcinogenesis, the deletion in 3p occurs before the myc gene is amplified. It is also of note that

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>K-ras p21</td>
<td>Careinoma</td>
<td>11</td>
<td>82</td>
<td>66</td>
</tr>
<tr>
<td>N-ras p21</td>
<td>Careinoma</td>
<td>11</td>
<td>0</td>
<td>(elevated expression equals ++ or +++ in this paper)</td>
</tr>
<tr>
<td>H-ras p21</td>
<td>Careinoma</td>
<td>11</td>
<td>0</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ras p21</td>
<td>Invasive</td>
<td>47</td>
<td>77*</td>
<td>100</td>
</tr>
<tr>
<td>RAP-5 and</td>
<td>m.situ CA</td>
<td>7</td>
<td>71*</td>
<td></td>
</tr>
<tr>
<td>Y13-259</td>
<td>Fibrocytic disease (246)</td>
<td>20</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>(mAb)</td>
<td>without hyperplasia</td>
<td>16</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>with hyperplasia (26)</td>
<td>10</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(i) without atypia</td>
<td>16</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(ii) with atypia</td>
<td>10</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ras p21</td>
<td>Careinoma</td>
<td>20</td>
<td>86</td>
<td>70</td>
</tr>
<tr>
<td>Y13-259</td>
<td>Fibrocytic disease</td>
<td>3</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>(mAb)</td>
<td>Fibrocytic disease</td>
<td>5</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ras p21</td>
<td>Careinoma</td>
<td>12</td>
<td>0</td>
<td>111</td>
</tr>
<tr>
<td>Western blotting</td>
<td>Breast CA pts</td>
<td>7</td>
<td>100</td>
<td>Elevated levels of ras p21 accompanied by high GTPase activity</td>
</tr>
<tr>
<td>Y13-259</td>
<td>Hormone Independent Breast cancer pts</td>
<td>6</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>(mAb)</td>
<td>Fibrocytomas</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Western blotting</td>
<td>Careinoma</td>
<td>54</td>
<td>69</td>
<td>60</td>
</tr>
<tr>
<td>Y13-259</td>
<td>Associated with progression and prognosis of the disease</td>
<td>54</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>(mAb)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the erbAB sequence has been localised to the 3p21-3p25 region which overlaps the deletion in SCLC, and it has been suggested that erbAB is possibly the recessive oncogene involved in SCLC (86).

In view of these wide ranging observations, no clear pattern for the genesis of SCLC has emerged. There is obviously a 3p deletion involved in the early stages of SCLC but this may also be seen in other lung cancers, and the erbB sequences may be the recessive oncogene that is involved in the early stages. In addition, the myc gene family has a role in the progression of SCLC but the reports vary on which member is important. Clearly the ras gene family is also integrated into this process and may correlate with the developmental process of lung cancers in general.

6. Breast cancer

6i. c-myc genetic alterations in breast cancer. This is one of the most comprehensively studied human diseases as over 500 breast cancer specimens have been investigated for alterations in their genetic structure or in their levels of expression of the c-myc gene (Table 1a). As yet, there is no evidence for amplification or re-arrangement of the N-myc gene and only one example of L-myc in an infiltrating ductal carcinoma (39). However, amplification, of the c-myc gene in malignant breast cancer has been found at varying frequencies by different investigators: six per cent (45), seventeen per cent (39), thirty-two per cent (87) and fifty-seven per cent (44). As the majority of these investigations were all carried out with similar probes using similar techniques, it does appear incongruous that such a wide range of c-myc amplifications has been found in breast cancer patients (6-57 per cent). The incidence of c-myc re-arrangements is usually very low (0-4 per cent) except in the paper by Bonilla et al. (44), who found 14 per cent; however, the clinical implications of the results of Bonilla et al are unknown, as the authors have not as yet published the survival data on these patients.

Initially, two comprehensive studies undertaken on c-myc alterations and breast cancer (39, 87) have come to very different conclusions. In the larger study, with 121 patients, a significant correlation (P<0.02) was found between the presence of a genetically altered c-myc gene in the tumour tissue and patients' age (87). In this study there were 40 patients who had either c-myc amplification or re-arrangements of the gene, and 29 (72.5 per cent) of these patients were over 50 years of age, thereby indicating a correlation between menopausal status and c-myc gene alterations. But this study indicated no correlation between c-myc gene alteration and oestrogen-progesterone-receptor status, tumour grade, or auxiliary lymph node metastasis. No data are available on the survival of these patients.

The smaller investigations with 41 patients had the benefit of follow up data (39). No association was found between c-myc alteration and patient age; however, the authors, have demonstrated a significant correlation between the altered c-myc gene and very poor short-term prognosis (P<0.02). It is of interest that Varley et al (39) also demonstrated that alterations of the neu gene (7 of 41; 17 per cent) correlated well with short-term prognosis in the same group of breast cancer patients (P<0.0002). In fact, in this study on 41 patients, none of the patients died or had a tumour recurrence who did not have an altered c-myc or neu gene. These findings implicate the activation of the c-myc gene in the progression of breast cancer, although whether associated with postmenopausal status (87) or with the more aggressive end stage disease (39) has still to be determined. Also the possibility of an interaction of an altered neu gene with the c-myc gene in the end stage disease has to be considered. One would have hoped that these conflicting results might have been resolved in the two papers published in 1988 (44, 45). However, the interpretation of these results became even more complex. Only six per cent of 100 carcinomas in one study (45) were found to have amplified c-myc gene, whilst in the other study Bonilla et al (44) found that 21 of their 37 (57 per cent) malignant tumour specimens had c-myc amplification. Although a high incidence of c-myc amplification was reported by Bonilla et al, no clinical correlations were found with the altered c-myc gene. Survival data on these patients are not yet available, and so it is not possible to correlate these data with prognosis.

A further development in the study on c-myc amplification in breast cancer comes from Guerin et al. (45), who found that only 6 per cent of the patients had c-myc amplification but 20 per cent of them had c-erbB-2/neu amplification. Significant correlations were found between c-erbB-2/neu amplification and the number of positive lymph nodes and oestrogen, progesterone receptor status. Lymph node involvement in breast cancers is considered to be the most important clinical factor in predicting recurrence (88). The correlation between erbB-2/neu oncogene and prognosis confirms earlier results of Slamon et al. (89) and Varley et al. (39).

C-myc alteration has not been found in many benign cancers: 0 of 5 (87), 1 of 17 (39), 0 of 6 (45), and 3 of 11 (44). Low levels of c-myc amplification were found in the one benign fibrocystic specimen that also showed histologically marked hyperplasia with foci of atypia, which is a pathology associated with increased risk of breast cancer (39). It is also of interest that in one specimen of cystosarcoma phylloides the c-myc gene was amplified and rearranged (44). These findings are based on very small numbers of benign breast tumours, but there are considerable clinical implications if one can identify premalignant breast lesions from alterations in the c-myc gene.

There are no clear cut conclusions that may be drawn concerning c-myc gene alterations in breast cancer. It appears that in certain studies (87) it stands out as an important gene in the progression of the disease and is intimately linked with the prognosis of the patients. In other studies (39) there appears to be an interaction between the c-myc and the neu oncogene in as much as all the patients who died had a
genetic alteration in one of these genes. Moreover a further complication of this story is that c-erbB-2/neu oncogene appears to have a much greater role in the course of the disease than the c-myc gene (45). Unless we consider that breast cancer patients treated in the different regions covered by these studies have very different diseases, which is highly unlikely, it is more probable that the role of c-myc gene alterations in breast cancer is very complex. Moreover, as yet the implication of c-myc amplification in benign breast tumours is unclear.

6ii. Elevated c-myc expression in breast cancers. It was previously considered that in all cases where there was c-myc amplification one would see a concomitant elevation of c-myc expression at the RNA level (90). However, this does not appear to be borne out in DNA and RNA studies on breast cancer. The numbers of malignant breast cancer patients reported with an over-expressed c-myc gene and also an amplified c-myc gene varies: 6 of 14 (87), 7 of 7 (39), 4 of 6 (45). The reason for these findings is unclear.

However, it appears that elevated c-myc expression observed at the RNA level does correlate with the prognosis of the patients with breast cancer. In a study of 41 breast cancer patients 10 were found to have an elevated c-myc RNA transcript of whom 5 have had a recurrence or have died; the remainder have only been followed up for 8-26 months (39). The results of the follow up of these patients will be of interest, as the remaining 5 patients may also be found to have a poor prognosis.

One of the most informative studies on c-myc expression in breast cancer has been by Guerin et al (45), who found that 45 out of 98 tumours had high levels of c-myc RNA and that this correlated with lymph node involvement (P<0.01). Moreover only eleven per cent of these tumours had c-myc amplification. They also reported that c-myc expression was elevated in lymph node metastases in 3 patients, as well as in the corresponding primary tumours. Therefore c-myc overexpression in breast cancer cannot be explained solely by amplification of the gene previously considered. In fact, two cases which had high levels of c-myc amplification (10 and >50 fold) had low levels of c-myc expression (45).

Using monoclonal antibodies to the c-myc protein, it was found that there were high levels of staining intensity in all malignant tumours, and also in the majority of the benign breast lesions analysed, whereas normal breast tissue exhibited very low levels of c-myc protein (91). The expression of c-myc was specifically studied in 198 specimens of fibrocystic disease compared to normal tissue using an improved immunohistochemical technique to detect c-myc by pretreating with neuraminidase (54). The results indicated that c-myc was not expressed in normal and epithelial cells in either ducts or lobes. However, high levels of staining were found in mucous metaplastic cells of epitheliosis and multiple papillomas, and the authors suggested that the elevated expression of c-myc in these cells may be involved in an early stage of malignant cell transformation.

A sensitive and quantitative ELISA has been developed for the c-myc oncoproteins (92) and it has been used to assess the level of c-myc in 24 breast cancer patients compared to normal tissue (61). It was demonstrated that all of the tumour specimens had considerably higher levels of c-myc oncoprotein than found in normal breast tissue from a patient with no evidence of breast cancer. A particularly intriguing piece of evidence was found in this investigation: a correlation was found between the extent of the tumour (T1 - T4) and c-myc expression in the tumour tissue (P<0.02), and also the level of c-myc expression in normal tissue adjacent to the breast cancer tumours correlated with the extent of the tumour.

These authors proposed that these high levels of c-myc expression found in normal tissue were perhaps due to ‘growth factors’ being released from nearby large tumours, and that the histologically normal tissues were not in fact molecularly normal. No correlation was found between the survival of these patients and elevated c-myc expression in the tumour tissue, and this maybe due to the fact that the majority of the patients had advanced disease.

6iii. Ras genetic alterations in breast cancer. In a large study of 104 breast cancer patients it was found that there was no evidence of rearrangements or amplification of the ras gene (93). However, recently in 24 breast tumours there was evidence of amplification in 6 tumours and 2 had an activating G-T mutation in codon 12 of H-ras (94). No amplification of the N-ras gene was found in 41 patients (39) (Table 1b).

The deletion of a normal cellular sequence is thought to unmask recessive mutations, and the analysis of lost genes on particular chromosomes using restriction fragment length polymorphisms (RFLP) has had rewarding results in a number of paediatric hereditary disorders and certain adult malignancies (11, 95, 96). The H-ras gene has a number of Bam H1 RFLP and loss of one of the H-ras 1 alleles on chromosome 11p was detected in 14 out of 51 (27 per cent) breast cancer patients who were constitutionally heterozygous for this locus (93). Even though loss of this allele did not change the level of p21 ras expression, it was found that the loss correlated with histological differentiation, lack of oestrogen and progestereone receptors and distal metastasis. Spandlos (94) also demonstrated that 2 out of 24 (8 per cent) of the breast tumour specimens had lost the H-ras 1 allele. The loss of H-ras 1 alleles in these tumour tissue specimens may indicate the existence of a regulatory sequence that is important in the initiation of breast cancer. Alternatively, the normal H-ras gene or another gene located near it may act as an onco-suppressor (97) (Table 1b).

6iv. Expression of the ras gene family in breast cancer. The expression of the ras gene family in breast cancer has been studied with immunohistochemical techniques using the
monoclonal antibodies Y13-259 (98) and RAP-5 (99), by Western Blotting, and by RNA hybridisation experiments (Table I). The specificity of the two main monoclonals for p21 ras has been debated and is still considered to be contentious (55, 100, 101, 102).

Spandidos and Aghantis (103) found that 12 out of 12 breast tumours over-expressed the H-ras mRNA compared to normal tissue using RNA dot hybridisation analysis, and in their follow up paper Aghantis et al (104) reported that elevated H-ras mRNA expression in 24 patients was associated with advanced histological types. In a Northern Blot analysis of mRNA from malignant breast tumours, 73 per cent were found to have elevated levels of H-ras, but K-ras and N-ras were at base line levels (93).

In another study a completely opposite finding was reported by Whittaker et al (105) regarding ras gene expression. They assessed the levels of c-myc, H, K and N-ras mRNA expression in 27 benign and 23 malignant breast cancers, and found that H-ras was only over-expressed in one specimen of a benign and in a malignant tumour compared to elevated expression in N-ras (65 per cent in carcinomas and 26 per cent in benign tumours) and K-ras (48 per cent in carcinoma and 15 per cent in benign tumours). These authors concluded from the analysis of these four oncogenes that there was a significant difference in oncogene expression between the benign and carcinoma specimens (P<0.01).

Expression of ras p21 was examined by RAP-5 monoclonal antibody by Horan-Hand et al (99), and it was found that 63 per cent of the malignant mammary tumours had increased levels of staining compared to 10 per cent of the benign tumours. The Y13-259 monoclonal was used by Aghantis et al (106) to ascertain the level of ras p21 expression in malignant and benign breast disease, and they reported that 83 per cent of the carcinomas and 42 per cent of cystic disease and 23 per cent of fibro-adenomas had moderate or above staining intensity but no clinical correlation was found in this study. In contrast to these results, a very different result was published by Tanaka et al (66). These authors used monoclonal antibodies which had been raised against peptides, two of them had homology with K-ras and N-ras, and the other with H-ras. In 10 breast cancer specimens none of them reacted with the H-ras monoclonal but 80 per cent reacted with the K-ras and N-ras monoclonals.

A comprehensive study using both RAP-5 and Y13-259 monoclonal antibodies to ras p21 was performed by Ohuchii et al (100) to determine the levels of expression in a range of benign and malignant breast tumours. Invasive carcinomas demonstrated enhanced levels of ras p21 expression; 77 per cent had >50% positive cells with RAP-5. The 46 fibrocystic specimens were subdivided into those with and without hyperplasia and further subdivided into those with and without atypia. The subgroup hyperplasia with atypia had higher levels of p21 expression than found in the other benign tumours; however, the levels were much lower than those found in the carcinomas. Analysis of the patients with a 15 year follow up indicated that there was a higher level of ras p21 expression in hyperplasia specimens from patients who subsequently developed breast cancer.

In contrast to the results, Candlish et al (107) found no significant differential staining in ras p21 expression between malignant and benign breast tumours, and normal breast tissue adjacent to the resection margin using the monoclonal antibody Y13-259.

Recently it has been demonstrated with the use of a direct binding liquid competition radioimmuno assay that 24 out of 28 breast tumours had higher levels of ras p21 than the average value found in fibro adenomas (70). These authors demonstrated that the higher levels of ras p21 were associated with post-menopausal patients.

The level of ras p21 protein in malignant, benign and normal breast tissue has been determined by Western Blotting analysis, and was found to be elevated in all 7 hormone responsive specimens analysed and in 5 out of 6 hormone independent specimens examined; however, low levels of p21 were found in the benign and normal tissue (108). Another study using Western Blotting analysis demonstrated that 37 out of 54 carcinomas (69 per cent) had elevated levels of ras p21 (60) and there was an association between increase in p21 in 60 per cent of the T1 and T2 tumours. A significant difference was also found between high ras p21 levels and a short disease-free interval, (P<0.05).

The results of these investigations indicate that the H-ras oncogene is important in the progression of malignant breast cancer, and that K-ras and N-ras may also play a major role. It is also clear that neither amplification nor rearrangement of the Ha-ras 1 gene is important. However, certain reports suggest that loss of one H-ras allele was significantly linked to parameters of tumour aggressiveness, (93), while in another study loss of one H-ras allele (14 of 65 informative patients) was correlated with paucity of the oestrogen receptor (109). However, Sheng et al (110) reported that the presence of variant alleles of H-ras 1 locus are not informative markers in breast cancer. The information on ras p21 expression in malignant breast tumours points to this gene having a role in the progression of the disease. The higher levels of p21 were identified in invasive tumours, in those that have metastasis and also in those that have a poor prognosis.

7 Colorectal Carcinomas

Cancer of the colon provides an excellent opportunity to study the progression of neoplasia, because most carcinomas appear to be derived from adenomas (111) and therefore specimens from different stages of the disease may be investigated. Both the ras and myc gene families have been analysed in these tumours, and results are now available from reasonably large scale studies with long-term follow up (36, 42). (Table IIa, b).

7i C-myc expression in colorectal carcinomas. Genetic
Table IIA. Review of ras and myc expression and genetic alterations in colon cancer.

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Tumour type</th>
<th>No. of patients</th>
<th>Percentage with amplification</th>
<th>Percentage with elevated expression</th>
<th>Clinical correlations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-myc (RNA)</td>
<td>Carcinoma</td>
<td>5</td>
<td>100</td>
<td>No clinical correlation reported</td>
<td></td>
<td>138</td>
</tr>
<tr>
<td>c-myc (RNA)</td>
<td>Carcinoma</td>
<td>6</td>
<td>0</td>
<td>Increased expression in these 6 tumours is accompanied by a parallel increase in expression of two G1 specific genes and S phase specific gene</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>c-myc (RNA)</td>
<td>Carcinoma</td>
<td>29</td>
<td>0</td>
<td>No c-myc amplification or rearrangements observed. C-myc more abundant in central portions of tumour than at periphery</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>c-myc (Southern)</td>
<td>Carcinoma</td>
<td>41</td>
<td>7</td>
<td>No clinical correlation found</td>
<td></td>
<td>112</td>
</tr>
<tr>
<td>c-myc (RNA)</td>
<td>Carcinoma</td>
<td>38</td>
<td>68</td>
<td>No correlation between c-myc expression and recurrence or patient survival</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>c-myc (mRNA)</td>
<td>Polyps</td>
<td>6</td>
<td>0</td>
<td>Pts with increased fos, myc and H-ras expression had poorer prognosis</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>(Southern)</td>
<td>Carcinoma metastasis</td>
<td>14</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-myc</td>
<td>Polyps (24)</td>
<td>2</td>
<td>0</td>
<td>Staining intensity greater in well differentiated tumours</td>
<td>140</td>
<td></td>
</tr>
<tr>
<td></td>
<td>adenomatous villous</td>
<td>11</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myel-6E10 (mAb)</td>
<td>Carcinoma</td>
<td>42</td>
<td>+++++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>moderate to well differentiated</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>poorly differentiated</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C-myc Myel-6E10 (mAb) and immunoblotting, Southerns.

No c-myc amplification or rearrangements found in these tumours, c-myc p62 detected by immunoblotting and immunohistochemistry gave similar results. Correlation between c-myc p62 and histological grades.
Field and Spandidos: ras and myc Oncogenes in Human Solid Tumours (Review)

Table II A continued

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Tumour type</th>
<th>No. of patients</th>
<th>Percentage elevated expression</th>
<th>Percentage with RAS mutations</th>
<th>Clinical correlations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-myc Myel-6E10 (mAb)</td>
<td>Carcinoma</td>
<td>100</td>
<td></td>
<td></td>
<td>c-myc expression in most colon CA. No correlations with differentiation, staging or survival</td>
<td>116</td>
</tr>
</tbody>
</table>

Table II B.

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Tumour type</th>
<th>No. of patients</th>
<th>Percentage elevated expression</th>
<th>Percentage with RAS mutations</th>
<th>Clinical correlations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-ras N-ras H-ras (oligonucleotides)</td>
<td>Carcinoma</td>
<td>27</td>
<td>37</td>
<td></td>
<td>In 5 of the 6 patients with K-ras mutations same mutation in benign and malignant sections of the tumour. No amplifications of any of the ras genes.</td>
<td>46</td>
</tr>
<tr>
<td>K-ras (RNase mismatch cleavage analysis)</td>
<td>Carcinoma</td>
<td>66</td>
<td>0</td>
<td>39</td>
<td>In 7 of 8 tumours originating in adenomas have mutant K-ras genes</td>
<td>47</td>
</tr>
<tr>
<td>K-ras N-ras H-ras (Adenoma 80)</td>
<td>Carcinoma</td>
<td>92</td>
<td>41</td>
<td>5</td>
<td>Ras gene mutations correlate in colon carcinogenesis, but not necessarily the first event as only 13% of class I adenomas have a mutation (only 1 adenoma had a N-ras mutation)</td>
<td>117</td>
</tr>
<tr>
<td>K-ras N-ras H-ras (class I)</td>
<td>40</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ras N-ras H-ras (class II)</td>
<td>19</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ras N-ras H-ras (class III)</td>
<td>21</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ras2 H-ras2 (oligonucleotides)</td>
<td>Adenoma</td>
<td>40</td>
<td>65</td>
<td>75</td>
<td>K-ras mutations occur early in colon carcinogenesis before change to aneuploidy</td>
<td>119</td>
</tr>
<tr>
<td>K-ras H-ras</td>
<td>Carcinoma</td>
<td>12</td>
<td>100</td>
<td></td>
<td>All the carcinomas had elevated K-ras expression over that found in normal tissue and six had H-ras elevated expression. All adenomas had elevated K-ras and H-ras gene transcripts</td>
<td>52</td>
</tr>
<tr>
<td>K-ras H-ras</td>
<td>4</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ras H-ras</td>
<td>4</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ras H-ras (RNA)</td>
<td>12</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ras</td>
<td>Polyps</td>
<td>6</td>
<td>17</td>
<td></td>
<td>No amplifications or rearrangement of H-ras or K-ras reported</td>
<td>53</td>
</tr>
<tr>
<td>K-ras</td>
<td>Carcinoma</td>
<td>14</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ras</td>
<td>metastasis</td>
<td>2</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ha-ras</td>
<td>Polyps</td>
<td>6</td>
<td>0</td>
<td></td>
<td>High levels of fos, myc and K-ras, H-ras expression correlated with poor prognosis</td>
<td></td>
</tr>
<tr>
<td>Ha-ras</td>
<td>Carcinoma</td>
<td>14</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Ha-ras | metastasis | 2 | 0 | | | | continue  

13
Table II continued

<table>
<thead>
<tr>
<th>ras p21</th>
<th>Polyps</th>
<th>4</th>
<th>Presence of ras p21 found in all stages of colon carcinogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y13-259 (mAb)</td>
<td>Carcinomas</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>ras p21</td>
<td>Carcinoma metastasis</td>
<td>17</td>
<td>53</td>
</tr>
<tr>
<td>Y13-259 (mAb) and immuno-blotting</td>
<td></td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>ras p21</td>
<td>Carcinoma</td>
<td>21</td>
<td>Presence of ras p21 found in all stages of colon carcinogenesis</td>
</tr>
<tr>
<td>Y13-259 (mAb)</td>
<td>Adenoma</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>ras p21</td>
<td>Carcinoma</td>
<td>6</td>
<td>(using their paper's staining intensity + or ++ equals elevated expression)</td>
</tr>
<tr>
<td>Y13-259 (MAb's)</td>
<td>Adenoma</td>
<td>6</td>
<td>Adenomas showed significantly greater staining intensity compared to carcinomas (P&lt;0.01)</td>
</tr>
<tr>
<td>ras p21</td>
<td>Carcinoma</td>
<td>6</td>
<td>Adenomas showed greater staining intensity than carcinomas using Y13-259, whereas RAP-5 bound to many types of cells, normal and neoplastic</td>
</tr>
<tr>
<td>Y13-259 (MAB's)</td>
<td>Adenoma</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

alterations in the form of c-myc amplifications and rearrangements are rare events in colon carcinoma, with only a few reported cases (53) in 1 of 2 colon metastases and (112), in 3 out of 45 colon carcinomas. Moreover, in a study of 29 cases of primary adenocarcinomas there was no evidence of c-myc amplification or rearrangements (113).

Elevated expression of the c-myc gene has been reported in about 70 per cent of all primary adenocarcinomas of the colon by Erismann's group (42, 113, 114). They demonstrated that the c-myc gene transcript was elevated about 5 fold greater than that found in normal mucosa. Similar results on c-myc RNA transcripts have been reported in smaller numbers of patients by Yokota et al (62) and Monnat et al (53).

However, the 40 month clinical follow up paper on the 38 patients with colon adenocarcinoma (42) provides evidence for no statistical correlation between patients with elevated levels of c-myc RNA and tumour recurrence or survival. These authors suggest that there is no obvious clinical value in measuring c-myc expression with respect to these patients' progress and that the expression of the c-myc gene is not an important factor in the late stages of tumorigenesis of the colon.

The myc-1-9E10 monoclonal antibody has also been used to analyse c-myc expression in colon tumours (64, 115, 116, 140). The reports in the literature concerning the c-myc nuclear cellular localisation in colon tumours using myc monoclonal antibody are uncertain. However, Jones et al (116) critically reviewed the use of this monoclonal and concluded that the staining pattern observed may be indicative of its real distribution at a tissue level (i.e. in both the cytoplasm and the nucleus) and that this oncprotein may have a more widespread distribution and may also have other functions not previously considered. Nevertheless, the conclusion drawn from the c-myc monoclonal data (116) indicates that the expression of this oncogene is increased in the majority of colorectal carcinomas but is unrelated to clinical behaviour and this is in agreement with c-myc mRNA data (42).

7ii. Ras genetic alterations in colon cancer. There is now considerable evidence for the involvement of ras gene mutations in the progression of colon carcinoma (46, 47, 117), whereas there is no evidence for amplification or rearrangement of the ras genes in these tumours (46, 53, 112).

The development of sensitive oligomer hybridization assays in conjunction with methods for selective amplification
of specific sequences has provided a method of reliably quantifying ras gene mutations in tumour specimens. Using these techniques approximately 40 per cent of colon tumours have been shown to have an activated ras gene (46, 117) and these results are in agreement with those reported using a RNase mismatch cleavage analysis (47). The majority of the activated ras genes had a mutation at codon 12 in the K-ras gene.

Moreover, these results indicate that the activated ras gene in colon carcinomas occurs relatively early in the development of these tumours. A strikingly high incidence of mutant K-ras genes were found in colon tumours originating in adenomas or polyps, 7 out of 8 (47) and 5 out of 6 (46). Although mutations in codon 12 of the K-ras gene occurred in certain incidences, it was found that N-ras activation also occurred in a villous adenoma and in one carcinoma (118), thereby indicating that one of these mutations was probably not associated with the initial or early event in the development of these tumours. This has important implications in the timing of genetic changes during the progression of colon cancer.

Burman and Loab (119) have also investigated the timing of ras mutations in colon cancer, using techniques of histological enrichment, cell sorting, DNA amplification and PCR followed by DNA sequencing. They found that in 40 carcinomas, 27 were aneuploid and 26 contained mutations in codon 12 of the K-ras 2 gene. Moreover 4 of the 12 adenomas were aneuploid and 9 had the same K-ras 2 mutations. These authors thereby suggested that the mutation in K-ras 2 pre-empted the change in ploidy status.

The possibility that constitutional differences in oncogene structure or expression may increase the chance of malignant transformation was investigated by Wyllie et al (120). They postulated that the chance of malignant transformation may be determined by additional genetic events apart from ras gene mutations to drive the cell into tumourigenesis. However, analysis of H-ras restriction fragments length polymorphisms (RFLPs) in patients with colorectal carcinomas showed that the frequency of rare alleles was not statistically different in these patients, compared to control groups.

An association between ras gene mutations and allelic deletions of chromosomes 5, 17 and 18 was investigated in adenomas and carcinomas from 172 specimens (117). The adenomas were divided into three classes representing different stages of the disease (increasing from Class I to III). The Class II and III adenomas contained ras mutations at the same frequency as carcinomas (50%), whereas in Class I only 13 per cent had ras mutations (P<0.001), mainly in the K-ras gene. Also none of the Class I adenomas had an allelic deletion on chromosome 5, whereas Class II and III had 29 per cent. These results indicate that ras mutations and allelic deletions in chromosome 5 occurred at earlier stages of the disease than deletion in 18q, which also precedes deletions in 17p. This is one of the first papers that provides evidence for the progressive accumulation of genetic alterations in carcinogenesis.

Recently a comprehensive survey of allelic losses has been undertaken with DNA markers from every nonacrocentric autosomal arm in 56 colon tumours and paired normal specimens (36). They found that the patients with greater than the median percentage of allelic deletions were more likely to develop a tumour recurrence (P<0.01) and were also more likely to die from their cancer (P<0.01). However, the incidence of ras gene mutations in this group of patients (i.e. greater than the median percentage of allelic deletions) was similar to that in the group of patients with less than the median value. The significance of multiple allelic losses (e.g. in tumour S141-A) is unclear, but the identification of this feature must make one more cautious in the interpretation of oncogene activation and allelic losses.

7iii. Ras oncogene expression in colon carcinoma. Elevated expression of H-ras and K-ras RNA transcripts was initially reported by Spandidos and Kerr (52). They demonstrated that elevated expression of one or both of these oncoproteins was elevated in all of the 4 colorectal polyps and in most of the thirteen adenocarcinomas of the colon analysed, compared to normal tissue, whereas elevated K-ras expression was seen in only 4 out of 14 adenocarcinomas and 1 out of 6 polyps; and elevated H-ras expression in 2 out of 14 carcinomas and in none of the polyps analysed by Monnat et al (53). Clearly these investigations are on small samples and need to be confirmed with prospective studies.

The p21 ras protein has been investigated in colon tumours in a number of studies using the monoclonal antibodies Y13 259 (55, 56, 121, 122) and with RAP-5 (67, 99). There has been some debate in the literature concerning the specificity of RAP-5 (55). However, Czerniak et al (67) did show a distinct difference in the negatively staining pattern of RAP-5 in benign colonic lesions and the positively staining carcinomas.

The results with Y13 259 monoclonal appear to contradict this as it was demonstrated that adenomas showed a consistently higher level of ras p21 expression by staining intensity than that found in carcinomas (P<0.01) and in normal tissue (P<0.002) (56) and these results are in agreement with Robinson et al (55). In contrast to this, Kerr et al (121) using Y13 259, reported that ras p21 was found in all tissues and was not restricted to any specific stage of colon carcinogenesis.

A direct binding liquid competition radioimmunoassay (RIA) has been used to quantify ras p21 in colon tumours (70) and 4 of the 5 tumours tested showed increased ras p21 in these tissues compared to normal. In two of these samples high levels of p21 were found in the adjacent normal tissue and it is postulated that the carcinoma cells provide a ‘factor’ that influences the expression of p21 with normal cells and that this may be important in the early transformation of the histologically normal cells. It is of interest to note that elevated expression of the c-myc gene was found in normal tissue adjacent to breast cancers using a c-myc ELISA (61). In both of these investigations it is possible that the histolo-
gical normal tissue is not molecularly normal and may be undergoing the initial stages of carcinogenesis.

The role of the *ras* and *myc* gene families in colonic carcinoma is still very uncertain. The c-*myc* gene may be overexpressed in certain carcinomas but as yet appears to have no particular association with the Staging of the disease. Little is known about this oncogene in the benign and premalignant stages of colon cancer. Nevertheless there may be a role for c-*myc* when its action is considered in conjunction with the *fos* and *ras* genes (53). In this study all the five deaths that occurred had elevated expression of *fos*, *myc* and H- or K-*ras* (*P*<0.01).

The *ras* gene family appears to be involved in the early stages of colon carcinoma, but it cannot as yet be correlated just with the initiating event, especially as the Class I adenomas in the paper of Vogelstein et al (114) had a significantly lower number of *ras* mutations than Class II or III adenomas. All the same, the evidence from immunohistochemical analysis, even though controversial, does point to an association between elevated *ras* p21 expression and benign colon tumours.

8. Cervix cancer

Cancer of the uterine cervix is the most prevalent cancer in under-developed countries (37) and it mainly affects women in lower socioeconomic groups.

A number of factors have been linked to the progression of this disease. Certain human papilloma virus DNA sequences have been found to be integrated into the cells of uterine cervix cancer cells and are transcribed (48) and the presence of these viruses in pre-neoplastic cervical lesions indicates a poorer prognosis (123). Recently certain oncogenes have also been implicated in the progression of the disease (38, 48, 49). In 154 patients with cervical cancer, 6 per cent had an amplified c-*myc* in stages I and II, compared to 49 per cent in stages III and IV. This indicates that c-*myc* amplification is associated with advanced disease (*P*<10^-5). Also elevated levels of c-*myc* expression are significantly more frequent in stages III and IV (*P*<10^-5). It was recognised by Riou and co-workers (38) that over-expression of the c-*myc* genes was found in some patients with stage I or II cervical cancer who subsequently had a relapse. They then analysed c-*myc* expression in 72 previously untreated patients with cervical cancer (Stage I or II) and found 25 (35 per cent) had high levels of expression. In a multi-variate analysis only c-*myc* over-expression and nodal status correlated with a risk of relaps, and in fact c-*myc* over-expression was the major prognostic factor (*P*=0.001).

This association between c-*myc* over-expression in the early stages of cervical cancer and a poor prognosis points very clearly to the involvement of this gene in the progression of the disease. In a smaller study Ocadiz et al (124) reported that 17 out of 35 (49 per cent) cases of cervical carcinoma had an amplified c-*myc* gene, and that 43% presented with both amplification and rearrangement of the gene. Riou and co-workers have also investigated the role of mutations in codon 12 in H-*ras* and also loss of H-*ras* heterozygosity in cervical cancer. No correlation was found between loss of H-*ras* heterozygosity and advanced stages of the disease but mutations in H-*ras* codon 12 correlated with cancers of a poor prognosis (*P*<0.01), (49). It is of interest that these authors also found that 4 out of 10 carcinomas which had a mutated H-*ras* gene also had a deletion of the H-*ras* gene on the other allele. This observation opens up a possibility that perhaps the loss of the H-*ras* gene on one allele contributed to the activation of the mutated H-*ras* gene on the other allele. Elevated expression of the ras p21 gene has been demonstrated in cervical carcinoma using the Y13-295 monoclonal antibody (125), and demonstrated a higher staining intensity in malignant cells than in the benign or premalignant lesions.

The combined results of H-*ras* and c-*myc* data in Riou's and co-workers investigations patients yielded a further interesting association, in as much as all the tumours with a mutated H-*ras* gene also had an amplified c-*myc* gene (49). This may indicate oncogene co-operation in the progression of this cancer which was similarly demonstrated by Land et al (126, 127) in an experimental model system where both *ras* and *myc* co-operated to transform the cell line.

9. Head and neck cancer

The incidence of head and neck cancer varies considerably worldwide. Oral cancer accounts for about 2 per cent of all malignancies in the western world where it accounts up to 40 per cent of all malignancies in India and South East Asia. When worldwide figures are computed for mouth and pharynx cancer, this group accounts for 6 per cent of all solid tumour malignancies (37). However, the prognosis for many of these patients is poor and even in the cases that have undergone successful surgery, many suffer severe degrees of dysfunction. The molecular mechanisms operating in neoplasia of the head and neck have not been investigated to the same extent as those in breast, colon or lung, but recent work has indicated that the ras and myc gene families and possibly erbB-1 and EGF may be involved (13, 40, 128-135).

9i. Myc expression and amplification in head and neck cancers. We have found evidence for elevated myc oncogene expression in 14 head and neck squamous cell carcinoma (SCC), from the tongue, floor of mouth, buccal mucosa, hypopharynx and larynx regions (13, 128, 129, 130). The increase in c-*myc* expression correlated with the stage of the disease, there was a significant difference between c-*myc* oncogene expression in TNM stages I and II and stage III (*P*<0.05), and also combined stages III and IV (*P*<0.05). In the combined stages III and IV it was found that there was a ten fold increase in c-*myc* expression over normal oral tissue (129). Amplification of c-*myc* and N-*myc* oncogenes has been reported in 3 out of 23 and 8 out of 23 oral carcinomas.
### Table III. Genetic alterations and elevated levels of expression of ras and myc gene families in human solid tumours, correlated with the patients' clinical outcome using specific statistical analysis*.

<table>
<thead>
<tr>
<th>Tumour site</th>
<th>Oncogene</th>
<th>Correlations with follow-up data</th>
<th>P</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>c-myc</td>
<td>Amplification and rearrangements correlated with poor prognosis</td>
<td>&lt;0.02</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>c-myc</td>
<td>Elevated oncoprotein expression does not correlate with prognosis</td>
<td>NSD</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>ras p21</td>
<td>Increased amount of H-ras p21 protein product associated with disease recurrence</td>
<td>&lt;0.05</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>ras p21</td>
<td>In 15 year follow up - higher levels of ras p21 in hyperplasia (benign), in patients who subsequently developed cancer of the breast</td>
<td>&lt;0.01</td>
<td>100**</td>
</tr>
<tr>
<td>Cervix</td>
<td>c-myc</td>
<td>c-myc over expression significantly associated with risk of relapse</td>
<td>&lt;0.001</td>
<td>38</td>
</tr>
<tr>
<td>Colon</td>
<td>c-myc</td>
<td>High levels of expression of these oncogenes correlated with poor prognosis</td>
<td>&lt;0.01</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>fos</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H, K-ras</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c-myc</td>
<td>No correlation between c-myc expression and recurrence or survival</td>
<td>NSD</td>
<td>42</td>
</tr>
<tr>
<td>Lung (SCLC)</td>
<td>N-myc</td>
<td>Elevated expression correlated with short survival</td>
<td>&lt;0.01</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>L-myc</td>
<td>No correlation between myc amplification and survival</td>
<td>NSD</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>N-myc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head and neck</td>
<td>c-myc</td>
<td>Elevated c-myc oncoprotein expression correlates with poor prognosis</td>
<td>&lt;0.02</td>
<td>40</td>
</tr>
<tr>
<td>Stomach</td>
<td>TGFα and</td>
<td>Carcinomas with synchronous expression of TGFα and H-ras correlated with poor prognosis</td>
<td>&lt;0.05</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>ras p21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* log rank test (59), Cox's proportional hazard model (58) and survival data compared by the Kaplan Meier method (57).
** Fisher's exact method.

respectively (134). These authors indicate that amplification of the myc gene is associated with advanced stages of the disease.

Recently we have analysed c-myc oncoprotein in SCC of the head and neck using an ELISA technique (92). In 44 specimens of SCC, the median level of c-myc oncoprotein expression in normal tissue adjacent to the resection site was 0.37 (range 0.16-1.01) pg c-myc/µg total protein (tp), while the median level of c-myc expression in tumour specimens was 0.77 (range 0.12-13.25) pg c-myc/µg tp. A significant difference was found between the survival of patients with low levels of c-myc expression and those with high levels of c-myc expression (P=0.02) (40). We have also demonstrated a correlation between elevated c-myc expression and prognosis using the myc 1-9E10 monoclonal antibody (135).

9ii. Ras genetic alterations in head and neck cancer. The expression of H-ras and K-ras are both elevated in SCC of the head and neck (128, 129) but no correlation was found between the expression of these genes and the progression of the disease.

However, using the monoclonal antibody Y13-259 to ras p21, Azuma et al (131) have demonstrated a relationship between increased expression of ras p21 and poor prognosis in 121 SCC of the head and neck (P<0.001). Moreover, correlations were reported between elevated ras p21 expression and histological grading and also with clinical staging. The TNM system of clinical staging in head and neck cancer is considered to be best prognostic indicator of the head and neck region (136).

In this study of ras p21 expression, Azuma et al (131) also included 44 specimens of oral leukoplakia which are considered to be potentially malignant lesions in approximately 5 per cent of cases, and therefore may represent a potentially
malignant clinical stage in oral cancer. None of these lesions when compared to normal tissue showed an increase in ras p21 expression.

There is little information on ras gene activation in the head and neck region. In a study on 20 oral SCC, point mutations were rare in H-, K- or N-ras genes in head and neck patients in Western Europe. (Chang, unpublished; Chang and Field, unpublished). Recently it has been reported in 2 oral cancer cell lines that there are mutations at codon 12 and 13 of H-ras and also concurrent amplification of c-erbB-1 and c-myc genes (132). The cell lines with these genetic alterations were established from a metastatic lymph node in the neck, the primary site being in the palate. This may be interpreted as probably the most advanced stage in multistep carcinogenesis and that multiple genetic defects have occurred during the development of this cancer. In the context of timing of these genetic alterations in oral cancer, clearly c-erbB-1 may correspond to an early event in tumourigenesis as demonstrated by Wong et al (137) in the hamster cheek pouch model system. They chemically induced oral carcinomas in this model system using DMBA and they demonstrated that the c-erbB-1 gene was amplified. A possible explanation for these results is that erbB-1 is involved in initiation events, ras genes are overexpressed throughout at the promotion and progression stages, and c-myc at the very late stages of tumour progression.

10. The significance of ras and myc gene families in the progression of human carcinomas

In this review it has been our intention to summarize the relevant work on the ras and myc oncogenes in the most common human tumours. Great progress has been made in this decade in determining that both ras and myc families are important in the progression of human neoplasia, but as yet we do not clearly understand the role of these genes in multistep carcinogenesis. As clearly stated earlier in this review, the number of investigations that contribute to our understanding of the timing of specific genetic effects is quite small. Only breast and colon tumours have clearly defined clinical stages of malignancy that have been studied to any great extent.

In a number of benign breast cancer patients with fibrocystic disease, the c-myc gene has been found to be overexpressed when measured by immunohistochemical techniques (54, 91) and by RNA hybridization analysis (105). However, the c-myc gene is not normally amplified or rearranged in benign breast tumours (45, 87), apart from one or two cases found by Varley et al (39) and Bonilla et al (44).

The ras p21 gene product also appears to be elevated in a large number of benign breast cancers as shown by Horan Hand et al (99), Aganantis et al (106), Ohuchi et al (100), and Whittaker et al (105), but these results were not corroborated by DeBortoli et al (108) and DeBiasi et al (70). One paper on p21 ras expression in benign breast tumours is very informative on the role of ras genes in early neoplasia (100). This study has followed up patients with fibrocystic disease for 15 years and found that the patients who subsequently developed breast carcinomas had high levels of p21 ras at the initial biopsy. It has been argued that the ras and myc genes co-operate in neoplasia, (126-127); it is therefore of interest that a significant difference in c-myc/ras gene expression was observed between benign and malignant breast tumours (105). It may thus be argued that there is evidence for the over-expression of ras and myc gene families at the initial stages of breast cancer.

In a number of adenomatous and villous polyps of the colon, elevated c-myc expression has been observed (25, 53), however, there is no evidence for amplification or rearrangement of this gene (25, 115).

There appears to be a very strong association between over-expression of the ras genes and benign colon cancers. Spandidos and Kerr (52) demonstrated that all four adenomas analysed had elevated K- and H-ras gene transcripts, and that the ras expression in these adenomas was elevated over that found in the colon carcinomas. This result is supported by the finding of Williams et al (56), who found that adenomas showed a significantly higher ras p21 staining intensity than carcinomas using the Y13-259 monoclonal antibody. A different result was found by Kerr et al (121), who reported ras p21 expression at all stages of colon cancers. The most striking evidence for the involvement of ras genes in colon tumour development comes from the work on ras mutations in adenomas (46, 47, 117). It is of specific note that the same K-ras mutations were seen in benign and malignant sections of colon tumours, but by subclassifying adenomas into classes I to III, it was found that the earliest stages (i.e. class I) had significantly fewer ras mutations than class II or III. These reports indicate that the ras genes are important in the initiation phase of colon cancer but it is possible that an unknown primary genetic event takes place beforehand.

A number of clinicopathological parameters associated with human cancers have been used to predict the prognosis of these patients. These may be grouped into patient factors, tumour factors and treatment factors; however, all are inter-linked and it is extremely difficult to dissect out any specific factors as being wholly responsible for the patients' prognosis. Tumour factors are those that have been used most widely in clinical correlations with oncogene studies, but the most useful parameter is the fate of the patient. A correlation between an oncogene's expression or genetic alteration with survival is one of the most valuable pieces of clinical information available, when risk of relapse is evaluated by Cox's proportional hazard model (58) or the rank log test (59), and survival rates computed by the Kaplan-Meier method (57). This information is not only valuable from a potentially prognostic view point, but it also provides information on the roles of a specific gene at the most aggressive stage of the disease.

Unfortunately, this type of data is not really available for
many investigations, but what published information is available is shown in Table III. C-myc genetic alterations are associated with poor prognosis in breast cancer (39), and overexpression of the c-myc gene is correlated with a risk of relapse in cervical carcinoma (38) and in SCC of head and neck (40). No correlation was found for c-myc over-expression in colon cancer (42). In SCLC opposite results pertaining to correlations between the N-myc expression and survival have been reported (43, 77).

There are few reports on ras p21 in human solid tumours that have correlated changes in expression with survival data using good statistical analysis. In breast cancer it has been shown by Clair et al (60) that elevated p21 ras expression correlates with disease recurrence, and Monnat et al (53) have demonstrated that high levels of c-myc, fos and H, K-ras expression correlate with poor prognosis in colon neoplasms. However, recently Vogelstein et al (36) have studied the allelotype, (polymorphic DNA markers in every nonacrocentric autosomal arm) in 56 colorectal carcinomas. They found that patients with above the median number of allelic losses had a worse prognosis. However, no correlation was found for the prevalence of ras gene mutations in the group of patients with a value over the median number of allelic deletions (P<0.001 Fisher exact test). In stomach carcinomas Yamamoto and Hattori (71) demonstrated that the synchronous expression of TGFα and Ha-ras p21 expression correlated with prognosis.

Reports in the early literature gave an erroneous impression that it might be possible to attribute a particular oncogene or anti-oncogene to certain clinical stages of neoplasia. However, the data reviewed in this paper highlight the difficulties involved in assigning either the ras or myc gene families to any specific stage in multistep carcinogenesis. The type of investigation now being undertaken requires an accurate sampling of the pathological specimens (i.e. to ensure that actual tumour tissue is being analysed) (46) and the use of scientifically evaluated probe banks for both oncogene and deletion mapping studies. There must also be good clinical follow-up over a meaningful length of time. Valid statistical analysis (Cox’s proportional hazard model or log rank test and survival rates computed by Kaplan-meier’s method) of these results can only be undertaken if a sufficiently large number of tumour specimens are available for study.

Despite the reservations outlined in this review, the advances in our understanding of oncogenes in the past decade have been considerable. In future it may be possible to envisage a “molecular index” of human carcinomas and in this way the full clinical potential of oncogene research could be realised.

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