[CANCER RESEARCH 49, 6324–6327, November 15, 1989]

Detection of Point Mutations in N-*ras* and K-*ras* Genes of Human Embryonal Rhabdomyosarcomas Using Oligonucleotide Probes and the Polymerase Chain Reaction[1]

M. R. Stratton,[2] C. Fisher, B. A. Gusterson, and C. S. Cooper

Section of Chemical Carcinogenesis [M. R. S., C. S. C.], Chester Beatty Laboratories, Institute of Cancer Research, Fulham Road, London SW3 6JB; Section of Pathology [B. A. G.], Haddow Laboratories, Institute of Cancer Research, 15 Cotswold Road, Belmont, Sutton, Surrey SM2 5NG; and Department of Histopathology [C. F.], Royal Marsden Hospital, Fulham Road, London SW3 6JJ, United Kingdom

ABSTRACT

Previous studies have demonstrated that genes of the *ras* family (H, K, and N) can be activated by point mutations at codons 12, 13, and 61. In the present study we have used oligonucleotide probes corresponding to these regions to assess the role of *ras* gene mutations in the genesis of human rhabdomyosarcoma. To increase the sensitivity of this method the appropriate regions of the three *ras* genes were first amplified using the polymerase chain reaction. The results show that 35% (5/14) embryonal rhabdomyosarcomas investigated contain mutations in the N-*ras* or K-*ras* genes. Thus *ras* gene mutation is implicated in the development of mesenchymal and embryonal tumors in addition to its previously documented role in epithelial and hematological neoplasia.

INTRODUCTION

In recent years recombinant DNA technology has allowed the isolation and characterization of several genes which are implicated in oncogenesis. For example, transfection of tumor DNA into NIH3T3 fibroblasts has revealed activated transforming genes in 10–20% of transformed cell lines and solid tumors (1–3). Most of these were either H-, K-, or N-*ras*, members of a gene family that had previously been encountered as oncogenes in acutely transforming retroviruses (4–8). Sequence analysis of transforming *ras* genes from human tumors showed that point mutations at codons 12, 13, or 61 commonly confer transforming activity upon the p21 *ras* gene products (see Ref. 9 for review). Point mutations introduced into other regions such as codons 59, 63, and 116 can also induce transformation in experimental models but these mutations have not yet been detected in human tumors (10, 11).

Although several oncogenes have been detected by this approach, the NIH3T3 transfection assay has several disadvantages as a method for investigating the epidemiology of *ras* gene activation in human tumors. Large amounts of high molecular weight DNA are required which may be difficult to obtain from substantial numbers of rare or small tumors. Moreover the technique is time consuming, labor intensive, and can be capricious in execution. Recently, however, technical methods have become available which require only limited amounts of DNA and which allow examination of large numbers of tumor specimens including material from histopathology archives. One of these, the RNase protection assay, detects single base pair mismatches between RNA/RNA or RNA/DNA hybrids formed *in vitro* from cloned *ras* gene segments and cognate sequences from tumors (12). Alternatively, DNA from tumors may be hybridized under stringent conditions to a panel of oligonucleotide probes each of which is complementary to a mutated homologue of codons 12, 13, or 61 of the *ras* genes (13). The sensitivity of both methods may be increased by amplifying the copy number of the target sequences several thousand fold using the polymerase chain reaction (14).

Use of the RNase protection assay and oligonucleotide probes has clarified the patterns and incidence of *ras* gene mutations in human tumors. Activated *ras* genes are found in both epithelial and hemopoietic tumors. For example most pancreatic carcinomas, approximately 50% of colon tumors, and 20–30% of cases of acute myeloid leukemia contain mutated *ras* genes (12, 15–18). However in some tumors such as carcinomas of the breast or ovary, *ras* gene activation appears to be a rare event (19, 20). The relevance of these differences to etiology or tumor biology remain unclear.

There remain, however, a number of other major tumor categories in which the role of *ras* gene mutation has not been determined. In particular there is little published data concerning the sarcomas, malignant tumors which recapitulate the differentiation of mesenchymal tissues such as fat, connective tissue, bone, and cartilage. Classified within this group are the rhabdomyosarcomas which are tumors differentiating towards striated muscle (21). Transfection of rhabdomyosarcoma DNA into NIH3T3 cells has previously demonstrated the presence of transforming homologues of N-*ras* and K-*ras* in a rhabdomyosarcoma cell line and primary tumor, respectively (1, 22). Using the polymerase chain reaction and oligonucleotide probes corresponding to wild type and mutant *ras* sequences we have therefore searched for *ras* gene mutations in a series of rhabdomyosarcomas, most of which were derived from paraffin-embedded histopathological material.

MATERIALS AND METHODS

Extraction of DNA from Paraffin-embedded Specimens. Rhabdomyosarcoma specimens were obtained mainly in the form of paraffin blocks from histopathology archives except in two cases where frozen tumors were available. Tumors were obtained from the Royal Marsden Hospitals, London and Surrey, St. Thomas' Hospital, London, Kingston Hospital, All Saints Hospital, Chatham, and West Wales General Hospital. The diagnosis of rhabdomyosarcoma was made by at least two pathologists on light microscopy of standard hematoxylin & eosin-stained sections and in most cases was confirmed by immunocytochemistry and/or electron microscopy. DNA was extracted from histopathology archive material using a previously described method with minor modifications (23). 25- × 20-μm sections were cut from paraffin blocks of formalin-fixed rhabdomyosarcoma specimens and incubated at 48°C in 1 ml of extraction buffer [100 mM Tris-HCl (pH 7.5), 40 mM NaCl, 10 mM EDTA] to which was added 100 μl 10% (w/v) SDS[3] and 20 μl of a 25 mg/ml solution of proteinase K. After 16–24 h a further 0.5 ml of extraction buffer, 50 μl of SDS and 10 μl of proteinase K were added and the extraction continued for 24 h. The solution was

Received 4/27/89; revised 8/7/89; accepted 8/15/89.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants from the Medical Research Council and Cancer Research Campaign.

[2] MRC Training Fellow.

[3] The abbreviations used are: SDS, sodium dodecyl sulfate; PCR, polymerase chain reaction; TAQ buffer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin; SSC, standard saline citrate (0.15 M sodium chloride:0.015 M sodium citrate, pH 7.4).

twice extracted with phenol, and subsequently with a 1:1 (v/v) mixture of phenol:chloroform and a 25:1 mixture of chloroform:isoamyl alcohol. After addition of sodium acetate to a final concentration of 0.3 M, nucleic acids were precipitated at −20°C for 16–24 h by addition of two volumes of ethanol. The precipitate was pelleted and redissolved in 100 μl of TE (10 mM Tris-HCl, 1 mM EDTA). The concentration of DNA was measured using a fluorometer.

PCR. 1 μg of DNA was made up to 97 μl of a solution containing TAQ buffer, 200 μM dATP, dTTP, dCTP, and dGTP and 1 μM each primer in a sterile 0.5-ml Eppendorf tube which was then heated at 94°C for 10 min. Three μl of TAQ buffer containing three units of TAQ polymerase (Cetus Corporation) were added and the whole mixture was overlayed with 100 μl of paraffin oil to prevent evaporation. The DNA was subjected to 40 cycles of the PCR on a Cetus/Perkin-Elmer DNA Thermal Cycler. Each cycle was composed of three phases; 55°C (annealing), 72°C (extension), and 94°C (denaturation) each for 2 min. The success of the PCR was assessed by running 10 μl of the final mixture on a 2% (w/v) agarose gel and visualizing the product as a 100–130 base pair band by staining with ethidium bromide. The PCR primers were 20mer oligonucleotides complementary to sequences flanking codons 12 and 61 of H-, K-, and N-*ras*.

Hybridization to Oligonucleotide Probes. Ten μl of the total PCR mixture was dotted onto three duplicate nylon filters (Hybond N, Amersham International) using a dot blotting apparatus. The DNA was denatured by immersing the filters in 0.5 M NaOH, 1.5 M NaCl for 2 min, neutralized in 1.5 M NaCl, 0.5 M Tris-HCl (pH 7.2), for 1 min, dried and fixed to the membrane by exposure to UV light for 3 min. Filters were prehybridized for 30 min at 55°C in 3 M tetramethyl ammonium chloride, 50 mM Tris-HCl (pH 7.5), 2 mM EDTA, 0.3% w/v SDS, 5× Denhardt's solution (100× Denhardt's is 2% w/v polyvinylpyrrolidone, 2% w/v bovine serum albumin, 2% w/v Ficoll) and 100 μg/ml sonicated, denatured, salmon testes DNA. Hybridization to radiolabeled oligonucleotide probes was carried out in a fresh sample of the same solution at the same temperature for 1 h. The filters were rinsed twice in 2× SSC at room temperature and washed for 30 min in 3 M tetramethyl ammonium chloride, 50 mM Tris-HCl, pH 7.5, 2 mM EDTA, 0.3% w/v SDS. The temperature of the wash varied from 59 to 65°C depending on the oligonucleotide probe used. Filters were exposed to autoradiographic film at −70°C for periods ranging from 1 to 16 h.

The probes used were 20mer oligonucleotides (except in the cases of K-*ras* codons 12 and 13 which were 19mers) complementary to wild type and mutant sequences at H-*ras* codons 12 and 61, K-*ras* codons 12, 13, and 61, and N-*ras* codons 12, 13, and 61. Five pM of each oligonucleotide probe was end labeled with [γ-^{32}P]dATP using T4 polynucleotide kinase and purified over Nensorb 20 columns. The unlabeled probes and PCR primers were generously donated by Dr. C. J. Marshall (Institute of Cancer Research). To confirm the presence of a *ras* gene mutation in a tumor, the PCR was repeated on a further aliquot of tumor DNA, the product run on a 2% w/v agarose gel and blotted onto nylon filters by capillary transfer in 20× SSC. Hybridization to oligonucleotide probes was carried out as described above.

RESULTS

The histopathology and the clinical details of the 21 cases of rhabdomyosarcoma used in this investigation are shown in Table 1. Most fall into the category of embryonal rhabdomyosarcoma, consistent with the higher frequency in the general population of this subtype compared to alveolar and pleomorphic tumors.

Up to 200 μg of DNA was extracted from the paraffin-embedded material and this showed the extensive degradation characteristic of DNA from this source (data not shown). DNAs were subsequently subjected to the PCR in order to amplify short DNA segments including codons 12 and 61 of the three *ras* genes. The success of the PCR was assessed by the presence of a 100–130 base pair ethidium bromide staining band on 2% (w/v) agarose gels or the presence of a signal when hybridized to a wild type probe. All but four of the DNAs amplified efficiently in the PCR and since these failed irrespective of the priming oligonucleotides used, it is likely that this was due to the presence of an inhibitor of TAQ polymerase. These four cases were not included in further analyses or in Table 1.

Table 1 *Rhabdomyosarcoma cases examined for ras gene mutations*

The clinical details, histological subtype, and *ras* gene mutations of the tumors studied are shown. Bracketed cases indicate two separate tumors from the same individual.

Case	Age and Sex	Site	Diagnosis	*ras* mutation
1	4F	Bladder	E[a]	K13Asp
2	2M	Calf	E	
	3M	Calf (rec)	E	
3	5M	Testis (rec)	E	N61Lys
4	11F	Parotid	E	
5	20M	Face	E	
	20M	Orbit (rec)	E	
6	15M	Testis	E	
	16M	PAN (rec)	E	
7	39F	Thigh	E	
8	3F	Tongue	E	
9	14F	Retroperitoneum	E	
10	16M	Testis	E	N13Arg
	19M	Testis (rec)	E	N13Arg
11	31M	Penis	E	
	34M	Penis (rec)	E	
12	2M	Testis	E	N12Ser
13	20M	Testis (rec)	E	K12Cys
14	29M	Abdomen	E	
15	18M	Chest (rec)	A	
16	12F	Perineum	A	
17	43M	Thigh	P	
18	62M	Thigh	P	
19	57M	Axilla	P	
20	41M	Testis	P	
21	54M	Buttock	P	

[a] E, embryonal; A, alveolar; P, pleomorphic; rec, recurrence; PAN, para aortic nodes.

To investigate the presence of mutations in genes of the *ras* family, the amplified DNAs were dotted onto nylon filters and hybridized to olignucleotide probes. An example of such an experiment is shown in Fig. 1. Replicate filters were hybridized to probes complementary to wild type and mutant sequences at N-*ras* codon 12/13. While most samples generated a strong signal on hybridization to wild type probe (Fig. 1*i*) only one case hybridized to a probe complementary to the N-*ras* codon 12 serine mutation (GGT→AGT) (Fig. 1*ii*) and a further case to a probe complementary to a codon 13 arginine mutation (GGT→CGT) (Fig. 1*iii*). Whenever a candidate *ras* mutation was detected on a dot blot, a fresh aliquot of tumor DNA was subjected to the PCR, run on a 2% (w/v) agarose gel and blotted onto a nylon filter by capillary transfer to confirm the result. We have found that this procedure often yields a lower background hybridization signal than direct application by dot blotting. Examples are illustrated in Figs. 2 and 3. In Fig. 2 replicate gel blots have been hybridized to N-*ras* codon 61 wild type (Fig. 2*i*) and lysine probes (CAA→AAA) (Fig. 2*ii*) while Fig. 3 shows replicate gel blots which have been hybridized to K-*ras* codon 12/13 wild type (Fig. 3*i*), codon 12 cysteine (GGT→TGT) (Fig. 3*ii*), and codon 13 aspartate (GGC→GAC) (Fig. 3*iii*).

Five *ras* gene mutations were found in this series of rhabdomyosarcoma (Table 1, Figs. 1, 2, and 3), three in N-*ras*, and two in K-*ras*. Examination of the clinical and pathological features of these cases reveals that all are rhabdomyosarcomas of embryonal type. Although there were no obvious correlations with the spectrum of histological appearances within this group, all five cases arose from the region of the genitourinary tract and none from the limbs or head and neck which constitute the other common sites of origin. In one case (Case 10, N-*ras* codon

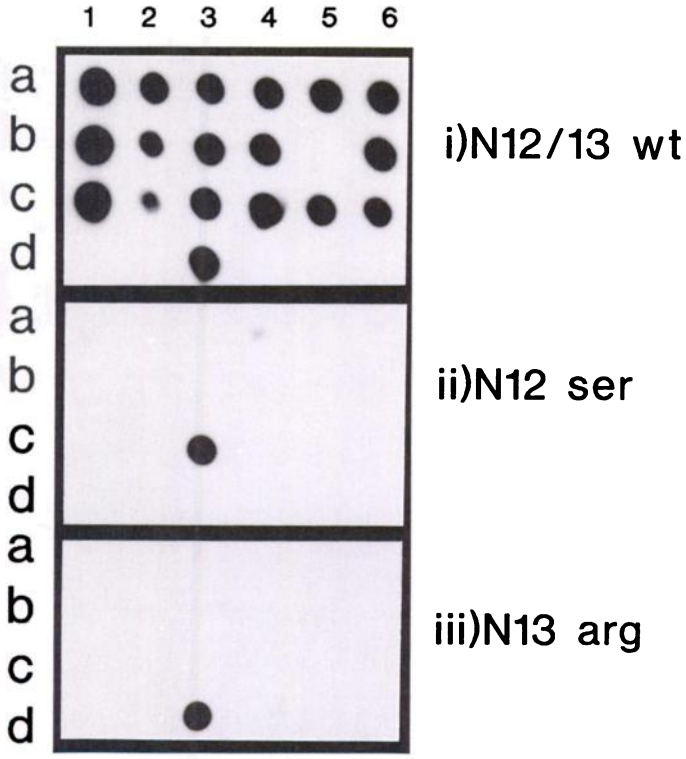

Fig. 1. Dot blots of PCR-amplified rhabdomyosarcoma DNAs hybridized to oligonucleotide probes complementary to wild type (*i*) and mutant (*ii* and *iii*) sequences at N-*ras* codons 12 and 13. Each *dot* represents a separate tumor or control (normal) DNA.

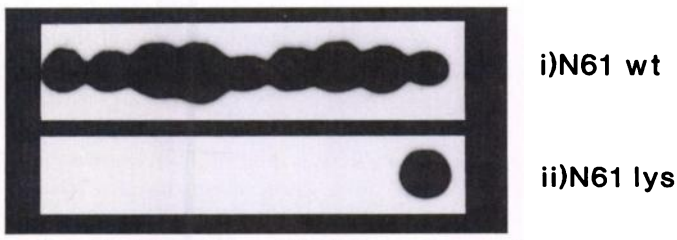

Fig. 2. Gel blots of PCR-amplified rhabdomyosarcoma DNAs hybridized to oligonucleotide probes complementary to wild type (*i*) and mutant (*ii*) sequences at N-*ras* codon 61.

13 Arg), we had access to material from a recurrence which was resected 3 years after the first presentation. In the intervening period the patient had undergone a course of chemotherapy. The recurrence contained the same mutated *ras* gene as the original tumor (Table 1).

DISCUSSION

Rhabdomyosarcomas are tumors which show features of skeletal muscle differentiation. Three histological subtypes are recognized (21). Most are embryonal rhabdomyosarcomas, primitive neoplasms which usually arise in the first and early second decades of life and which possess many properties similar to other embryonal tumors such as neuroblastoma, medulloblastoma, and retinoblastoma. Alveolar rhabdomyosarcoma appears slightly later, usually during the second decade, and is characterized by the presence of broad bands of dense fibrous tissue which separate aggregates of tumor cells. Finally, the rare pleomorphic rhabdomyosarcoma is usually a tumor of adults containing large, bizarre cells. The majority of primary rhabdomyosarcomas arise from the head and neck region, the limbs and around the genitourinary tract. Although

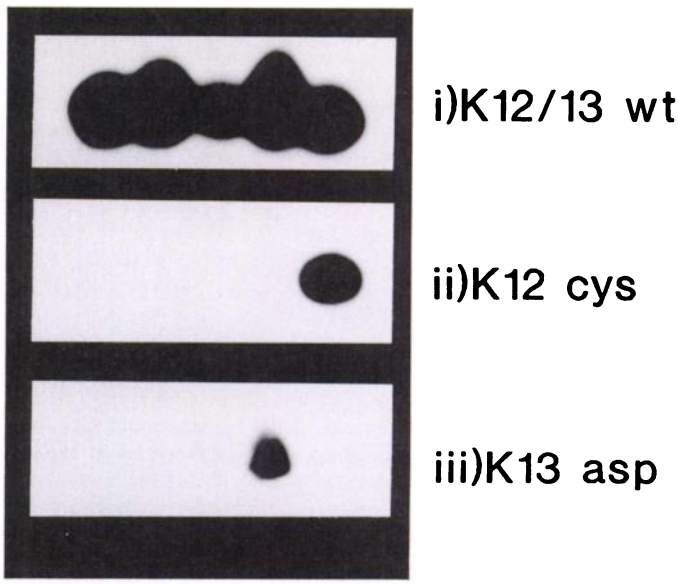

Fig. 3. Gel blots of PCR-amplified rhabdomyosarcoma DNAs hybridized to oligonucleotide probes complementary to wild type (*i*) and mutant (*ii* and *iii*) sequences at K-*ras* codons 12 and 13.

these sarcomas are highly malignant lesions and usually produce distant metastases, the prognosis has improved in recent years with the development of effective chemotherapeutic regimes.

We have examined a series of rhabdomyosarcomas for the presence of transforming mutations in H-, K-, and N-*ras* which might be involved in oncogenesis. The study shows that *ras* genes are mutated at the positions examined in approximately 35% (5/14) of embryonal rhabdomyosarcomas. This is therefore the first category of sarcoma or embryonal tumor identified in which activation of *ras* to transforming activity is a common event. The mutations in our series are in both N- and K-*ras* and are spread over many codons. This pattern differs from that found in colon carcinomas, pancreatic tumors and acute myeloid leukemia, where the reported activating mutations tend to be clustered at a single position of a particular *ras* gene (12, 15–18). It is, however, more reminiscent of the pattern described in thyroid neoplasms where mutations are spread over the H-, K-, and N-*ras* genes (24).

All the *ras* gene mutations detected in this report and in previous NIH 3T3 transfection studies (1, 22) were in embryonal rhabdomyosarcomas rather than alveolar or pleomorphic tumors. Since the latter two groups constitute a minority of cases both in the population and in our series, a more extensive study will be required to determine whether *ras* activation is really specific to the embryonal tumors. Nevertheless, this finding is reminiscent of observations on human lung carcinomas (25, 26) in which K-*ras* activation occurs frequently in adenocarcinomas but is rare in other non-small cell neoplasms. Within the group of embryonal rhabdomyosarcomas there appears to be no correlation between *ras* gene activation and histological features such as the degree of differentiation although we have noted that all our cases of *ras* mutation and in addition the previously reported cell line RD, are embryonal rhabdomyosarcomas arising from the genitourinary tract.

In the single case of recurrence of a tumor containing a mutated *ras* gene, the later specimen contained the same mutation as the earlier one. In the period between the two biopsies, the patient had been treated by chemotherapy. Farr *et al.* (17) have reported that in acute myeloid leukemia, recurrence after

the disease has been put into remission by combination chemotherapy commonly lacks the *ras* gene mutation present in the earlier specimen. This has been attributed to the presence of multiple clones within the tumor, presumably with differing chemosensitivity. We shall also be carrying out studies on additional cases of recurrent rhabdomyosarcoma in order to investigate whether in some cases this is true for rhabdomyosarcoma. Finally, three of our cases of *ras* mutation (Cases 1, 5, and 12) and one of the cases previously studied by transfection (1) were in children under 5 years of age. These results therefore indicate that etiological factors implicated in the induction of *ras* gene point mutations may be active during early childhood.

Three other types of genetic change have previously been reported in human rhabdomyosarcoma; loss of DNA segments on chromosome 11 (27, 28), a consistent translocation between chromosomes 2 and 13 (29), and occasional amplification of *myc* genes (30–32). The relationship between different genetic events in rhabdomyosarcoma warrants further investigation. For example in colonic neoplasms, activating mutations in *ras* genes commonly coexist with loss of putative tumor suppressor elements on chromosomes 17 or 18 (33). Therefore in this neoplasm, the two forms of genetic alteration appear to represent independent and cooperative steps in tumorigenesis. A similar relationship between *ras* activation and loss of the putative tumor suppressor gene on chromosome 11 may exist in embryonal rhabdomyosarcomas. Conversely these changes could represent mutually exclusive pathways to oncogenesis. To clarify this point we are preparing a further study in which deletion of chromosomal segments on chromosome 11 will be investigated in parallel with *ras* gene mutation.

ACKNOWLEDGMENTS

The authors would like to thank the consultant staff at the many hospitals which furnished us with tumor specimens and S. Moss and S. Williams for their technical assistance.

REFERENCES

1. Pulciani, S., Santos, E., Lauver, A. V., Long, L. K., Aaronson, S. A., and Barbacid, M. Oncogenes in solid human tumours. Nature (Lond.), *300:* 539–542, 1982.
2. Murray, M. J., Shilo, B-Z., Shih, T., Cowing, D., Hsu, H. W., and Weinberg, R. A. Three different human tumour cell lines contain different oncogenes. Cell, *25:* 355–361, 1981.
3. Perucho, M., Goldfarb, M., Shimizu, K., Lama, C., Fogh, J., and Wigler, M. Human-tumour-derived cell lines contain common and different transforming genes. Cell, *27:* 467–476, 1981.
4. Parada, L. F., Tabin, C. J., Shih, C., and Weinberg, R. A. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus *ras* gene. Nature (Lond.), *297:* 474–478, 1982.
5. Santos, E., Tronick, S., Aaronson, S., Pulciani, S., and Barbacid, M. T24 human bladder carcinoma oncogene is an activated form of the normal homologue of BALB- and Harvey-MSV transforming genes. Nature (Lond.), *298:* 343–347, 1982.
6. Hall, A., Marshall, C. J., Spurr, N. K., and Weiss, R. A. Identification of transforming gene in two human sarcoma cell lines as a new member of the *ras* gene family located on chromosome 1. Nature (Lond.), *303:* 396–400, 1983.
7. Shimizu, K., Goldfarb, M., Suard, Y., Perucho, M., Li, Y., Kamata, T., Feramisco, J., Stavnezer, E., Fogh, J., and Wigler, M. H. Three human transforming genes are related to the viral *ras* oncogenes. Proc. Natl. Acad. Sci. USA, *80:* 2112–2116, 1983.
8. Der, C., Krontiris, T., and Cooper, G. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the *ras* genes of Harvey and Kirsten sarcoma viruses. Proc. Natl. Acad. Sci. USA, *79:* 3637–3640, 1982.
9. Bos, J. L. The *ras* gene family and human carcinogenesis. Mutation Res., *195:* 255–271, 1988.
10. Walter, M., Clark, S. C., and Levinson, A. D. The oncogenic activation of human p21*ras* by a novel mechanism. Science (Wash. DC), *233:* 649–652, 1986.
11. Fasano, O., Aldrich, T., Tamanoi, F., Taparowsky, E., Furth, M., and Wigler, M. Analysis of the transforming potential of the human H-*ras* gene by random mutagenesis. Proc. Natl. Acad. Sci. USA, *81:* 4008–4012, 1984.
12. Forrester, K., Almoguera, C., Han, K., Grizzle, W. E., and Perucho, M. Detection of high incidence of K-*ras* oncogenes during human carcinogenesis. Nature (Lond.), *327:* 298–303, 1987.
13. Bos, J. L., Verlan-de-Vries, M., Jansen, A. M., Veeneman, G. H., van-Boom, J. H., and van der Eb, A. J. Three different mutations in codon 61 of the human N-*ras* gene detected by synthetic oligonucleotide hybridization. Nucleic Acid Res., *12:* 9155–9163, 1984.
14. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H., and Arnheim, N. Enzymatic amplification of B-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anaemia. Science (Wash. DC), *230:* 1350–1354, 1985.
15. Bos, J. L., Fearon, E. R., Hamilton, S. R., Verlaan-de-Vries, M., van Boom, J. H., van der EB, A. J., and Vogelstein, B. Prevalence of *ras* mutations in human colorectal cancers. Nature (Lond.), *327:* 293–297, 1987.
16. Almoguera, C., Shibata, D., Forrester, K., Martin, J., Arnhem, N., and Perucho, M. Most human carcinomas of the exocrine pancreas contain mutant c-K-*ras* genes. Cell, *53:* 549–554, 1988.
17. Farr, C. J., Saiki, R., Erlich, H. A., McCormick, F., and Marshall, C. J. Analysis of *ras* gene mutations in acute myeloid leukaemia by polymerase chain reaction and oligonucleotide probes. Proc. Natl. Acad. Sci. USA, *85:* 1629–1633, 1988.
18. Bos, J. L., Verlaan-de-Vries, M., van der Eb, A. J., Janssen, R., Delwel, B., Lowenberg, B., and Colly, L. P. Mutations in N-*ras* predominate in acute myeloid leukaemia. Blood, *69:* 1237–1241, 1987.
19. van't Veer, L. J., Hermens, R., van den Berg-Bakker, L. A. M., Cheng, N. C., Fleuren, G. J., Bos, J. L., Cleton, F. J., and Schrier, P. I. *ras* oncogene activation in human ovarian carcinoma. Oncogene, *2:* 157–165, 1988.
20. Rochlitz, C. F., Scott, G. K., Dodson, J. M., Liu, E., Dollbaum, C., Smith, H. S., and Benz, C. C. Incidence of activating *ras* oncogene mutations associated with primary and metastatic human breast cancer. Cancer Res., *49:* 357–360, 1989.
21. Enzinger, F. M., and Weiss, S. W. Soft Tissue Tumours. The C. V. Mosby Company, Ed. 2, 1988.
22. Marshall, C. J., Hall, A., and Weiss, R. A. A transforming gene present in human sarcoma cell lines. Nature (Lond.), *299:* 171–173, 1982.
23. Goelz, S. E., Hamilton, S. R., and Vogelstein, B. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. Biochem. Biophys. Res. Commun., *130:* 118–126, 1985.
24. Lemoine, N. R., Mayall, E. S., Wyllie, F. S., Farr, C. J., Hughes, D., Padua, R. A., Thurston, V., Williams, E. D., and Wynford-Thomas, D. Activated *ras* oncogenes in human thyroid cancers. Cancer Res., *48:* 4459–4463, 1988.
25. Rodenhuis, S., van de Wetering, M., Mooi, W. J., Evers, S. G., van Zandwijk, N., and Bos, J. L. Mutational activation of the K-*ras* oncogene; a possible pathogenetic factor in adenocarcinoma of the lung. N. Engl. J. Med., *317:* 929–935, 1987.
26. Rodenhuis, S., Slebos, R. J. C., Boot, A. J. M., Evers, S. G., Mooi, W. J., Wagenaar, S. S., van Bodegom, P. C., and Bos, J. L. Incidence and possible clinical significance of K-*ras* oncogene activation in adenocarcinoma of the human lung. Cancer Res., *48:* 5738–5741, 1988.
27. Koufos, A., Hansen, M. F., Copeland, N. G., Jenkins, N. A., Lampkin, B. C., and Cavenee, W. K. Loss of heterozygosity in three embryonal tumours suggests a common pathogenetic mechanism. Nature (Lond.), *316:* 330–334, 1985.
28. Scrable, H. J., Witte, D. P., Lampkin, B. C., and Cavenee, W. K. Chromosomal localization of the human rhabdomyosarcoma locus by mitotic recombination mapping. Nature (Lond.), *329:* 645–647, 1987.
29. Wang-Wuu, S., Soukup, S., Ballard, E., Gotwals, B., and Lampkin, B. Chromosomal analysis of sixteen human rhabdomyosarcomas. Cancer Res., *48:* 983–987, 1988.
30. Mitani, K., Kurosawa, H., Suzuki, A., Hayashi, Y., Hanada, R., Yamamoto, K., Komatsu, A., Kobayashi, N., Nakagome, Y., and Yamada, M. Amplification of N-*myc* in a rhabdomyosarcoma. Gann, *77:* 1062–1065, 1986.
31. Garson, J. A., Clayton, J., McIntyre, P., and Kemshead, J. T. N-*myc* oncogene amplification in rhabdomyosarcoma at release. Lancet, *1:* 1496, 1986.
32. Tsuda, H., Shimosato, Y., Upton, M. P., Yokota, J., Terada, M., Ohira, M., Sugimura, T., and Hirohashi, S. Retrospective study on amplification of N-*myc* and c-*myc* genes in pediatric solid tumors and its association with prognosis and tumor differentiation. Lab. Invest., *59:* 321–327, 1988.
33. Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M., and Bos, J. L. Genetic alterations during colorectal-tumor development. N. Engl. J. Med., *319:* 525–532, 1988.

Detection of Point Mutations in N-*ras* and K-*ras* Genes of Human Embryonal Rhabdomyosarcomas Using Oligonucleotide Probes and the Polymerase Chain Reaction

M. R. Stratton, C. Fisher, B. A. Gusterson, et al.

Cancer Res 1989;49:6324-6327.