Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 9228–9232, December 1987
Medical Sciences

RAS gene mutations in acute and chronic myelocytic leukemias, chronic myeloproliferative disorders, and myelodysplastic syndromes

(oncogene/oligomers/tumorigenicity assay)

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Communicated by Janet D. Rowley, August 20, 1987

ABSTRACT **We report on investigations aimed at detecting mutated *RAS* genes in a variety of preleukemic disorders and leukemias of myeloid origin. DNA transfection analyses (tumorigenicity assay) and hybridization to mutation-specific oligonucleotide probes established *NRAS* mutations in codon 12 or 61 of 4/9 acute myelocytic leukemias (AML) and three AML lines. Leukemic cells of another AML patient showed *HRAS* gene activation. By using a rapid and sensitive dot-blot screening procedure based on the combination of *in vitro* amplification of *RAS*-specific sequences and oligonucleotide hybridization we additionally screened 15 myelodysplastic syndromes, 26 Philadelphia chromosome-positive chronic myelocytic leukemias in chronic or acute phase, and 19 other chronic myeloproliferative disorders. A mutation within *NRAS* codon 12 could thus be demonstrated in a patient with idiopathic myelofibrosis and in another with chronic myelomonocytic leukemia. Moreover, mutated *NRAS* sequences were detected in lymphocytes, in granulocytes, as well as in monocytes/macrophages of the latter case.**

Recent evidence arising from several different approaches has given strong support to the view that neoplastic cells develop from normal progenitors as a consequence of changes in a set of cellular genes, called protooncogenes (1, 2). Of the 40–50 oncogenes presently known, the *RAS* genes belong to the best-studied sequences. This gene family is composed of the *HRAS*, *KRAS*, and *NRAS* genes encoding homologous proteins (p21) that have the biochemical property of binding guanine nucleotides and exhibit intrinsic GTPase activity and whose cellular localization is at the inner surface of the plasma membrane (1). Though the precise function(s) of the *RAS*-encoded proteins is not as yet known, they have been implicated with the transduction of receptor-mediated external signals into the cell.

Cloning and sequence analyses of oncogenic versions of *RAS* genes have revealed that the mechanism of activation involves a single base substitution that alters an amino acid of the corresponding p21 protein and thus decreases associated GTPase activity (1, 3, 4). Point mutations have been demonstrated in either codon 12, 13, or 61 of *RAS* genes (1, 5, 6). Moreover, in all cases investigated thus far, the mutations turned out to be specific for the tumor cells and were not found in normal cells of the respective patient. Activation of *RAS* genes has been detected in a variety of different neoplasias with variable frequencies. By far, the highest incidence (25–50%) has been reported in acute myelocytic leukemia (AML) (6–8).

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To gain further information on the biological significance of *RAS* mutations in leukemogenesis, we investigated the occurrence of *RAS* gene mutations in AML compared to a broad spectrum of other preleukemic and leukemic disorders involving the myeloid lineage. For this purpose we used, in addition to DNA transfection analyses (tumorigenicity assay), a dot-blot screening procedure based on a combination of *in vitro* amplification of *RAS*-specific sequences and hybridization to mutation-specific oligonucleotide probes (9–11).

MATERIAL AND METHODS

Patients. *AML.* We investigated nine primary AML cases (seven children, two adults) representing French–American–British (FAB) types M_2, M_3, and M_4 as well as three established cell lines—namely, THP-1 [monoblastic (12), provided by S. Tsuchiya (Tohoku University School of Medicine, Sendai, Japan)], Rc2a [myelomonocytic (13), obtained from P. Tetteroo (Red Cross Blood Transfusion Service, Amsterdam)], and KG-1 [myeloblastic (14), provided by H. Koeffler (University of California at Los Angeles School of Medicine, Los Angeles)].

Chronic myelocytic leukemia (CML). Twenty-six Philadelphia chromosome (Ph)-positive CML cases were analyzed, all of whom were molecularly characterized by a *ABL/BCR* rearrangement. Ten patients were in chronic state (lasting 3–47 months), 2 were in lymphoid state, and 14 were in myeloid blast crisis.

Myelodysplastic syndrome (MDS). We investigated 15 patients (aged 45–71 yr) with *de novo* MDS (lasting from 2 to 107 months) comprising various types according to the FAB classification (15): 3 refractory anemias (RA), 5 RA with ring sideroblasts (RARS), 2 RA with excess blasts (RAEB), and 2 chronic myelomonocytic leukemias (CMML); moreover, we included 2 cases of the 5q− syndrome (16). Except for the latter cases and the CMML patients (normal karyotypes), no cytogenetic data were available. All patients showed <1% blasts in peripheral blood; in RA, RARS, and 5q− syndrome cases, <5% blasts were observed, and in RAEB and CMML cases, <20% blasts were observed in bone marrow.

Abbreviations: AML, acute myelocytic leukemia(s); CML, chronic myelocytic leukemia; MPS, myeloproliferative syndrome(s); MDS, myelodysplastic syndrome(s); RA, refractory anemia(s); RARS, RA with ring sideroblasts; RAEB, RA with excess blasts; CMML, chronic myelomonocytic leukemia(s); ET, essential thrombocythemia; IMF, idiopathic myelofibrosis; PV, polycythemia vera; FAB, French–American–British; Ph, Philadelphia chromosome.

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Myeloproliferative syndrome (MPS). Nineteen chronic myeloproliferative disorders comprising the following subtypes were analyzed: four patients with essential thrombocythemia (ET), six cases of idiopathic myelofibrosis (IMF), and nine patients with polycythemia vera (PV). Patients' age ranged from 38 to 80 yr and, at the time of molecular analysis, MPS had lasted from 1 to 131 months. Cytogenetic analysis was performed in four IMF patients and showed no chromosomal aberrations. Cytogenetic data were not available from the other cases.

Cell Samples. After informed consent, bone marrow cells (AML, MDS, MPS) or peripheral blood cells (MPS, CML) were taken from respective patients.

In one CMML patient we separated various cell fractions obtained from peripheral blood specimens—namely, lymphocytes, granulocytes, as well as monocytes/macrophages according to standard techniques (17–19). Slides were prepared from each cell fraction for evaluation of purity by May/Grünwald/Giemsa and Sudan black B stainings and revealed >90% purity for each sample. Moreover, we took a skin biopsy from this patient's right forearm for DNA analysis.

Transfection Assays. The tumorigenicity assay is based on the cotransfection of 3T3 fibroblasts with tumor DNA and a dominant drug-resistant selectable marker, pRSVneo; this is followed by G-418 selection and injection of resulting 3T3 colonies into nude mice (20). A detailed description of the protocol used by us has been published (21). Rat liver DNA was used as a negative control for transfection analyses.

Southern Blot Analyses. Cellular DNAs were digested with restriction endonucleases (Boehringer Mannheim), electrophoresed on a 0.7% agarose gel, blotted, and hybridized as described (21). Inserts of the following *RAS* probes were used: pEJ [*HRAS*; ref. 22], p640 (*KRAS*; ref. 23), and 0.9-kilobase (kb) *Pvu* II fragment B (*NRAS*; ref. 24).

Oligonucleotide Synthesis and Hybridization. The oligonucleotides (20-mers) were prepared by one of us (J.L.) by means of an asynchronous simultaneous synthesis strategy (25) using the solid-phase phosphite triester method. Wild-type sequences were synthesized according to refs. 26–30. A complete list of oligomers used for the analyses of *RAS* gene mutations is available on request. Labeling and separation of kinased probes followed a published protocol (31). DNA was digested with *Pst* I (Boehringer Mannheim) to detect mutations in the *NRAS* gene and fractionated by electrophoresis in 0.6% agarose. The gel was treated as described (32) to denature the DNA *in situ* and to immobilize the DNA by drying the gel. Hybridization and washing conditions adapted to different oligomers were described by Janssen *et al.* (31).

Polymerase Chain Reaction. DNA amplification *in vitro* was performed as described by Saiki *et al.* (9). The modified method used by us has been published (31). A complete list of amplimers (oligomers used for the chain elongation) is available on request. The polymerase chain-elongation reaction was started by the addition of 0.5 μl (1 unit) of cloned Klenow polymerase (Pharmacia). Routinely we perform 15 rounds of amplification with an outer set of amplimers; this is followed by 15 rounds of amplification using an inner set of amplimers (10).

Dot-Blot Hybridization. Five nanograms of amplified DNA was spotted onto Nylon filters (GeneScreen*Plus*; New England Nuclear). Filters were prehybridized, hybridized to oligomer probes, washed as described (31), and finally exposed to Kodak XAR films at −70°C using intensifying screens.

RESULTS

In a series of DNA transfection analyses using the tumorigenicity assay, we observed a tumor induction in nude mice in four of seven AMLs and all three AML cell lines investigated (Table 1). Primary and/or secondary tumors were tested for the presence of *RAS* genes (*HRAS*, *KRAS*, and *NRAS*). All tumors contained human *NRAS* sequences (Table 1). To unravel the mode of *RAS* gene activation, we hybridized respective gels to synthetic oligonucleotides representing possible mutations within *NRAS* codons 12, 13, and 61. Indeed, each case could be traced back to a specific point mutation in codon 12 or 61 (Table 1, Fig. 1). Moreover, we could establish that different transfectants derived from a single cell line or primary AML contained identical mutated *NRAS* sequences (data not shown).

The analysis for the presence of mutated *RAS* genes by means of hybridization of mutation-specific oligonucleotide probes to genomic DNA has been considerably improved by including an *in vitro* amplification step of *RAS* sequences (9, 10). As a result, mutated *RAS* oncogenes can now be detected with a dot-blot screening procedure (11, 31). By using this method, we confirmed the results described above by analyzing the cell lines and AML as well as their respective transfectants (Fig. 2). Moreover, we included two additional AML in this study and demonstrated a *HRAS* gene mutation in codon 12 in AML case 9 (Table 1). Taken together these results indicate that in AML (*i*) a high proportion of cases contains activated *RAS* sequences and (*ii*) mutations in *NRAS* predominate in this type of leukemia. These conclusions are in agreement with recent data obtained by us and others (6–8).

Though *KRAS* activation has been reported previously (8), patient 9 of the present study exhibits mutated *HRAS*

Table 1. *RAS* gene mutation in AML

DNA source	Tumorigenicity assay, no. of tumors/ no. of experiments*	*RAS* gene mutated	Oligomer hybridization		
			Codon	Nucleotide	Amino acid
THP-1	3/2	*NRAS*	12	GGT → GAT	Gly → Asp
Rc2a	14/8	*NRAS*	12	GGT → GTT	Gly → Val
KG-1	4/3	*NRAS*	12	GGT → GTT	Gly → Val
AML 1	2/1	*NRAS*	12	GGT → GAT	Gly → Asp
AML 2	2/1	*NRAS*	61	CAA → CAT/C	Glu → His
AML 3	1/1	*NRAS*	61	CAA → CAT/C	Glu → His
AML 4	2/1	*NRAS*	61	CAA → CGA	Glu → Arg
AML 5	0/1	None			
AML 6	0/1	None			
AML 7	0/1	None			
AML 8	NT	None			
AML 9	NT	*HRAS*	12	GGC → GTC	Gly → Val

*In each experiment transfected cells were injected into both flanks of a single mouse; thus up to two tumors could develop per experiment. NT, not tested.

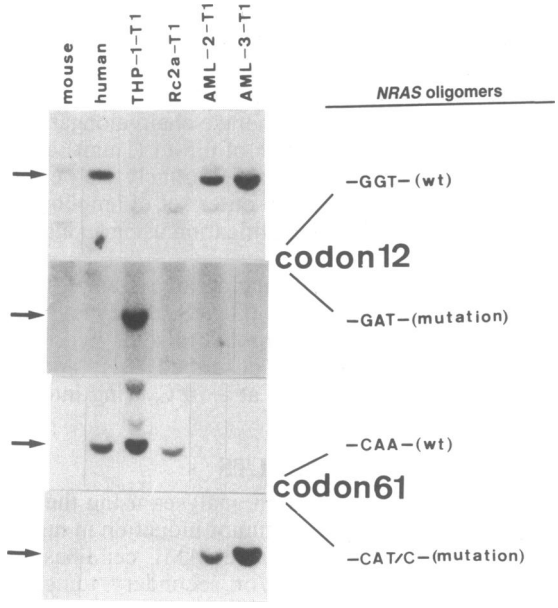

FIG. 1. Hybridization of *NRAS*-specific synthetic oligomers to genomic DNA of transfectants obtained from AML cell lines THP-1 and Rc2a as well as AML-2 and AML-3. The DNA was digested with *Pst* I, electrophoresed, and hybridized *in situ* to oligomer probes representing wild-type (wt) or mutated sequences of *NRAS* codons 12 and 61. N-12 probes hybridize to 3.0-kb *Pst* I fragments and N-61 probes hybridize to 4.2-kb *Pst* I fragments (arrows). Rc2a-T1 DNA contains a codon 12 mutation not detectable by the oligomer probe used in this experiment (see Table 1).

sequences. Thus, *RAS* gene mutations in AML are not restricted to *NRAS* but, rather, can afflict all members of this gene family.

Since the dot-blot screening procedure proved to be sensitive and rapid (ref. 11; this report) we subsequently focused on this technique in the analysis of myelodysplastic and myeloproliferative disorders. Investigation of 26 Ph-positive CML in chronic or acute phase gave no indication for mutated *RAS* genes (Table 2). This result is comparable with DNA transfection studies that likewise detected activated *RAS* sequences in CML very infrequently (33).

Analyses of 19 other chronic myeloproliferative disorders and 15 MDS (Table 2) revealed that the majority of those

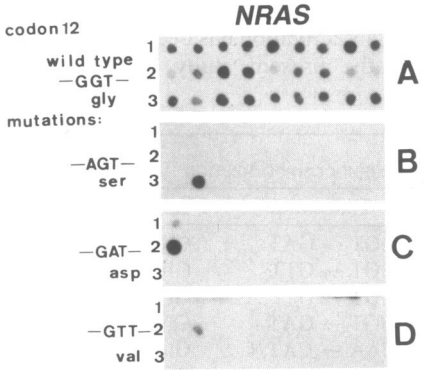

FIG. 2. Hybridization of mutation-specific oligomers to *in vitro* amplified DNA using N-12 amplimers. Five nanograms of the DNAs was spotted to GeneScreen*Plus* and hybridized to oligomers representing wild-type *NRAS* sequences present in all DNAs tested (*A*) and mutation-specific *NRAS* sequences (*B–D*). DNAs were obtained from AML 1–9 (lane 1); THP-1, Rc2a, KG-1, and MDS cases 1–6 (lane 2); MDS cases 7–15 (lane 3). Point mutations in *NRAS* codon 12 are detected in MDS case 8 (CMML, *B*), AML-1 and THP-1 (*C*), as well as cell line Rc2a (*D*).

Table 2. Patients screened for mutated *RAS* gene by dot-blot analyses

Diagnosis	No. of cases	Mutated *RAS* gene
MDS	15	
5q− syndrome	2	Negative
RA	3	Negative
RARS	5	Negative
RAEB	3	Negative
CMML	2	1 case: *NRAS* codon 12 GGT → AGT, Gly → Ser
CML	26	
Chronic phase	10	Negative
Lymphoid blast crisis	2	Negative
Myeloid blast crisis	14	Negative
MPS	19	
ET	4	Negative
PV	9	Negative
IMF	6	1 case: *NRAS* codon 12 GGT → GAT, Gly → Asp

cases contained no point mutations in codon 12, 13, or 61 of the *RAS* gene family. However, we observed two exceptions: a patient suffering from chronic myelomonocytic leukemia and another with IMF both exhibited *NRAS* sequences mutated in codon 12 (Table 2). We emphasize that the latter patients did not differ by clinical or laboratory criteria from the other patients with respective disorders and did not convert to AML since these analyses have been carried out (2 months).

The CMML patient was a 56-yr-old male diagnosed 6 months prior to molecular analysis. Bone marrow samples revealed 18% blasts, whereas no blasts were observed in peripheral blood. However, molecular analyses by the dot-blot technique also detected activated *NRAS* sequences in peripheral blood samples. We therefore decided to investigate separated cell fractions obtained from peripheral blood of this patient (Fig. 3). Indeed, a mutation in *NRAS* codon 12 could be established in lymphocyte, granulocyte, as well as monocyte/macrophage samples. In the monocyte fraction hybridization to the mutation-specific oligomer appears to give a stronger autoradiographic signal than the wild-type sequences. This difference is in all likelihood due to inadequate DNA spotting and was not observed in an additional experiment. Skin tissue obtained from this patient lacked this type of *RAS* gene mutation. Thus, a constitutional activation of *NRAS* sequences could be ruled out for this CMML case.

The IMF patient characterized by *NRAS* gene activation was a 63-yr-old male suffering from IMF for 30 months. Though his bone marrow showed 3% blasts, peripheral blood samples were free from blasts by morphological criteria but scored positive by molecular analyses (not shown). Unfortunately this patient refused further molecular analyses of separated blood cell fractions.

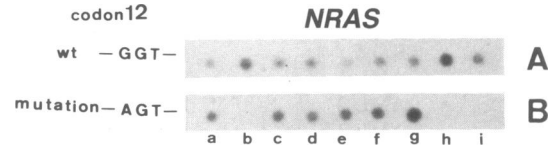

FIG. 3. Detection of mutated *NRAS* sequences in different hematopoietic cell lineages of a CMML patient. Five nanograms of amplified DNA obtained from the patient's peripheral blood (a), skin (b), bone marrow (c), an additional peripheral blood sample (d), lymphocytes (e), granulocytes (f), or monocytes/macrophages (g) as well as bone marrow cells from two other MDS patients (h and i) was hybridized to oligomers representing wild-type (*A*) and mutation-specific *NRAS* sequences (*B*).

DISCUSSION

In this study we have investigated the frequency of *RAS* gene mutations in a broad spectrum of preleukemic and leukemic disorders of the myeloid lineage. In agreement with recent reports (6–8) we detected an activation of *RAS* genes in a high proportion of AML. One striking observation is that mutations are predominantly found in *NRAS* but only rarely in *KRAS* (8) or *HRAS* genes (this report). In contrast to oncogene activations that appear to be characteristic for a specific neoplasia (e.g., *ABL/BCR* rearrangement in Ph-positive CML), *RAS* gene mutations are observed in a variety of different tumors at a frequency of 5–15% (1, 5). In this respect AML appears to be exceptional since up to 40% of the cases investigated thus far show *NRAS* mutations. This frequent *NRAS* activation seems to be a characteristic feature of AML and is not observed in other premalignant or leukemic entities of the myeloid (present report) or lymphoid lineage (34). Notably, none of 14 myeloid CML blast crises resembling AML by morphological and immunological criteria exhibited mutated *RAS* versions. Since *NRAS* mutations are common for different AML subtypes defined by morphological (FAB classification) or cytogenetic criteria, it appears to be unlikely that the *NRAS* gene is involved in a stage-specific differentiation pathway. As yet, the biological meaning of a *RAS* gene mutation in the multistep pathway of leukemogenesis remains to be elucidated. However, preliminary evaluation of the clinical data of our AML patients characterized by either the presence or absence of *NRAS* mutations shows no significant differences.

Activated *RAS* genes have not been exclusively observed in malignancies—e.g., mutated versions of *HRAS* are involved in the generation of benign skin papillomas of the mouse (35, 36). In this context it seems to be interesting that we demonstrate *NRAS* gene activations in two disorders that—despite their propensity to evolve into overt leukemia—are not reckoned to neoplasia *sensu stricto*. Moreover, in both patients we detected a mutated *NRAS* version in peripheral blood, though the morphological examination of respective cells failed to reveal a transformed phenotype. The more detailed investigations in the CMML patient revealed that three major hematopoietic cell lineages—i.e., lymphocytes, granulocytes, as well as monocytes/macrophages—all contain a mutated *NRAS* gene and thus may originate from a pluripotent stem cell.

Evidence of the underlying clonality of hematopoiesis in MDS has previously been established by cytogenetic, glucose-6-phosphate dehydrogenase, or, more recently, restriction fragment length polymorphism analyses (37–41). Along this line, mutated *RAS* sequences may constitute a further molecular/genetic marker in studying the extent of affected cell lineages in MDS, MPS, or AML patients.

One major problem in the management of MDS and MPS patients is the difficulty of predicting individually when, if at all, the disease will progress into overt leukemia (42). In the context of the high proportion of AML characterized by a *RAS* gene mutation, it would be of significant clinical value to know if an *NRAS* mutation in a MDS or MPS patient will predict an impending final transformation. The dot-blot screening procedure used in this investigation will considerably facilitate testing of this hypothesis by systematically investigating *RAS* sequences in MDS and MPS patients from the first clinical sign throughout further progression of the disease. Such studies have been initiated in our institutions.

After completion of this manuscript, a high frequency (40%) of *KRAS* mutations was reported in colorectal cancers (43, 44). Moreover, based on DNA transfection analyses (tumorigenicity assay), Hirai *et al.* (45) reported on identical mutations affecting *NRAS* codon 13 in three of eight MDS patients.

We thank Drs. E. Kleihauer, B. Kubanek, and H. Heimpel for continuous support, Dr. A. Wölpl for cell separations, and H. Barro for help with preparation of the manuscript. This research was supported by grants from the Deutsche Forschungsgemeinschaft.

1. Varmus, H. E. (1984) *Annu. Rev. Genet.* **18,** 553–612.
2. Land, H., Parada, L. F. & Weinberg, R. A. (1983) *Science* **222,** 771–778.
3. McGrath, J. P., Capon, D. J., Goeddel, D. V. & Levinson, A. D. (1984) *Nature (London)* **310,** 644–649.
4. Gibbs, J. B., Sigal, I. S., Roe, M. & Scolnick, E. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5704–5708.
5. Pierce, J. H., Eva, A. & Aaronson, S. A. (1986) in *Clinics in Hematology (Acute Leukemia)*, eds. Gale, R. P. & Hoffbrand, A. V. (Saunders, London), Vol. 15, pp. 573–596.
6. Bos, J. L., Toksoz, D., Marshall, C. J., Verlaan-de Vries, M., Veeneman, G. H., van der Eb, A. J., van Boom, J. H., Janssen, J. W. G. & Steenvoorden, A. C. M. (1985) *Nature (London)* **315,** 726–730.
7. Needleman, S. W., Kraus, M. H., Srivastava, S. K., Levine, P. H. & Aaronson, S. A. (1986) *Blood* **67,** 753–757.
8. Bos, J. L., Verlaan-de Vries, M., van der Eb, A. J., Janssen, J. W. G., Delwel, R., Löwenberg, B. & Colly, L. P. (1987) *Blood* **69,** 1237–1241.
9. Saiki, R., Scharf, S., Faloona, F., Mullis, K., Horn, G., Erlich, H. A. & Arnheim, N. (1985) *Science* **230,** 1350–1353.
10. Embury, S. H., Scharf, S. J., Saiki, R. K., Gholson, M. A., Golbus, M., Arnheim, N. P. & Erlich, H. A. (1987) *N. Engl. J. Med.* **316,** 656–661.
11. Verlaan-de Vries, M., Bergaard, E. M., van den Elst, H., van Boom, J. H., van der Eb, A. J. & Bos, J. C. (1986) *Gene* **50,** 313–320.
12. Tsuchiya, S., Yamabe, M., Kobayashi, Y., Konno, T. & Tada, K. (1980) *Int. J. Cancer* **26,** 171–176.
13. Bradley, T. R., Pillington, G., Garson, M., Hodgson, G. S. & Kraft, N. (1982) *Br. J. Haematol.* **51,** 595–604.
14. Koeffler, H. P. & Golde, D. W. (1978) *Science* **200,** 1153–1154.
15. Bennett, J. M., Catovsky, D., Daniel, M. T., Flandrin, G., Galton, D. A. G., Gralnick, H. R. & Sultam, C. (1982) *Br. J. Haematol.* **51,** 189–199.
16. Van den Berghe, H., Vermaelen, K., Mecucci, C., Barbieri, D. & Tricot, G. (1985) *Cancer Genet. Cytogenet.* **17,** 189–255.
17. Boyum, A. (1986) *J. Clin. Lab. Invest.* **21,** 31–35.
18. Edelson, P. J. & Cohn, Z. A. (1976) in *In Vitro Methods in Cell-Mediated and Tumor Immunity*, eds. Bloom, B. R. & David, J. R. (Academic, New York), pp. 330–342.
19. Thompson, J. S., Overlin, V., Severson, C. D., Parsons, T. J., Herbick, J., Strauss, R. G., Burns, C. P. & Claas, F. H. J. (1980) *Transplant. Proc.* **12,** 26–31.
20. Fasano, O., Birnbaum, D., Edlund, L., Fogh, J. & Wigler, M. (1984) *Mol. Cell. Biol.* **4,** 1695–1705.
21. Janssen, J. W. G., Steenvoorden, A. C. M., Losekoot, M. & Bartram, C. R. (1987) *Oncogene* **1,** 175–179.
22. Goldfarb, M. P., Shimizu, K., Perucho, M. & Wigler, M. H. (1982) *Nature (London)* **296,** 404–409.
23. McCoy, M. S., Toole, J. J., Cunningham, J. M., Chan, E. H. & Lowry, D. R. (1983) *Nature (London)* **302,** 79–81.
24. Hall, A., Marshall, C. J., Spurr, N. K. & Weise, R. A. (1983) *Nature (London)* **303,** 396–400.
25. Seliger, J., Ballas, K., Herold, A., Kotschi, U., Lyons, J., Eisenbeiss, F., Sinha, N. D. & Talwar, G. P. (1986) *Chem. Scr.* **26,** 569–577.
26. Fasano, O., Taparowsky, E., Fiddes, J., Wigler, M. & Goldfarb, M. (1983) *J. Mol. Appl. Genet.* **2,** 173–180.
27. McGrath, J. P., Capon, D. J., Smith, D. J., Chen, E. Y., Seeburg, P. H., Goeddel, D. V. & Levinson, A. D. (1983) *Nature (London)* **304,** 501–506.
28. Taparowsky, E., Shimizu, K., Goldfarb, M. & Wigler, M. (1983) *Cell* **34,** 581–586.
29. Sekiya, T., Fushimi, M., Hori, H., Hirohashi, S., Nishimura, S. & Sugimura, T. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4771–4775.
30. Hall, A. & Brown, R. (1985) *Nucleic Acids Res.* **13,** 5255–5268.
31. Janssen, J. W. G., Lyons, J., Steenvoorden, A. C. M., Seliger, H. & Bartram, C. R. (1987) *Nucleic Acids Res.* **15,** 5669–5680.
32. Bos, J. L., Verlaan-de Vries, M., Jansen, A. M., Veeneman,

G. H., van Boom, J. H. & van der Eb, A. J. (1984) *Nucleic Acids Res.* **12**, 9155–9163.
33. Eva, A., Tronick, S. R., Gol, R. A., Pierce, J. H. & Aaronson, S. A. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4926–4930.
34. Rodenhuis, S., Bos, J. L., Slater, R. M., Behrendt, H., van't Veer, M. & Smets, L. A. (1986) *Blood* **67**, 1698–1704.
35. Balmain, A., Ramsden, M., Bowden, G. F. & Smith, J. (1984) *Nature (London)* **307**, 658–660.
36. Roop, D. R., Lowy, D. R., Tambourin, P. E., Strickland, J., Harper, J. R., Balaschak, M., Spangler, E. F. & Yuspa, S. H. (1986) *Nature (London)* **323**, 822–824.
37. Nowell, P. C. (1982) *Cancer Genet. Cytogenet.* **5**, 265–278.
38. Prchal, J. T., Throckmorton, D. W., Carrol, A. J., Fuson, E. W., Gams, R. A. & Prchal, J. F. (1978) *Nature (London)* **274**, 590–591.
39. Raskind, W. H., Tirumali, N., Jacobson, R., Singer, J. & Adamson, J. W. (1984) *Blood* **63**, 1318–1323.
40. Abkowitz, J. L., Fialkow, P. J., Niebruge, D. J., Raskind, W. H. & Adamson, J. W. (1984) *J. Clin. Invest.* **73**, 258–264.
41. Kere, J., Ruutu, T. & de la Chapelle, A. (1987) *N. Engl. J. Med.* **315**, 499–503.
42. Koeffler, H. P. (1986) *Semin. Hematol.* **23**, 284–299.
43. Bos, J. L., Fearsson, E. R., Hamilton, S. R., Verlaan-de Vries, M., van Boom, J. H., van der Eb, A. J. & Vogelstein, B. (1987) *Nature (London)* **327**, 293–297.
44. Forrester, K., Almoguera, C., Hau, K., Grizzle, W. E. & Perucho, M. (1987) *Nature (London)* **327**, 298–303.
45. Hirai, H., Kobayashi, Y., Mano, H., Maru, Y., Omine, M., Mizoguchi, H., Nishida, J. & Takaku, F. (1987) *Nature (London)* **327**, 430–432.