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Genetics

β_2-Microglobulin gene mutations: A study of established colorectal cell lines and fresh tumors

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ABSTRACT **The technique of single-strand conformation polymorphism (SSCP) was used to screen a series of 37 established colorectal cell lines, 22 fresh tumor samples, and 22 normal DNA samples for mutations in the β_2-microglobulin gene. Exon 1 (including the leader peptide sequence) and exon 2 were screened separately. Six of 37 colorectal cell lines and 1 of 22 fresh tumors were shown to contain mutations, whereas no mutations were detected in the normal DNA samples. Sequencing of these mutations showed that an 8-bp CT repeat in the leader peptide sequence was particularly variable, since 3 of the cell lines and one fresh tumor sample have deletions in this region. In the related cell lines, DLD-1 and HCT-15, two similar mutations were identified, a C → A substitution in codon 10 and a G → T mutation in the splice sequence of intron 1. Expression of β_2-microglobulin was examined using a series of monoclonal antibodies in an ELISA system. Reduced expression correlated with a mutation in one allele of β_2-microglobulin, whereas loss of expression was seen in instances where a line was homozygous for a mutation or heterozygous for two mutations.**

The HLA class I molecules are composed of two noncovalently associated glycoproteins. The larger 44-kDa heavy chain is the product of the HLA-A, -B, and -C genes on chromosome 6, and the smaller 12-kDa chain is the product of the β_2-microglobulin (β_2M) gene on chromosome 15. A complex of these two glycoproteins with peptide formed from degraded endogenous proteins is expressed at the cell surface to enable the peptide to be presented to specific receptors on the surface of cytotoxic T cells (1). Several studies have shown that some tumors lack cell surface expression of HLA class I molecules and so indicate that this is one mechanism by which tumor cells escape immune recognition by cytotoxic T cells. Loss of HLA heavy chain surface expression has been demonstrated by immunohistochemical studies in colorectal adenocarcinomas (2, 3). In addition, these and other studies (4) have shown loss of reactivity with antibodies that recognize β_2M. Lack of HLA class I expression can be explained by specific mutations of the β_2M gene, as in the case of a G → C point mutation in the initiator ATG sequence in the Burkitt lymphoma cell line, Daudi (5); a double deletion in the 5′ flanking region and a segment of intron 1 in the melanoma cell line FO-1 (6); and a C → A point mutation in codon 10 and an 11-bp deletion at the beginning of exon 2 in a clone from the colorectal cell line HCT-15 (7).

The purpose of this study was to screen a large series of established colorectal cell lines, fresh colorectal tumors, and normal DNA samples for β_2M mutations and, where changes are indicated, to sequence these to identify the mutations involved. The expression of β_2M was also examined by ELISA and compared with the data on β_2M mutations.

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MATERIALS AND METHODS

Cell Lines and DNA Extraction. Most of the colorectal cell lines have been documented (8). Others reported elsewhere include HCT-116 (9), COLO 320DM (10), SW620 and SW480 (11), VACO4S and VACO10MS (12), and HRA19 (13). Cell lines used for control purposes were the Burkitt lymphoma Daudi (14) and the normal Epstein–Barr virus-transformed B-cell line Bristol-8 (Searle). Twenty-two normal DNA samples were used from the 4AOH series of cell lines (15). Biopsies of fresh colorectal tumors were obtained at the time of surgery, snap-frozen in liquid nitrogen, and cryopreserved until DNA was extracted. DNA extraction was performed either by Nucleon II kit extraction (Scotlab, Coatbridge, Scotland) or by using an Applied Biosystems model 340A nucleic acid extractor.

PCR. PCR was performed in 50 μl containing all four NTPs (each at 200 μM), 0.2 μM of the appropriate primer combination as shown in Fig. 1, 1 unit of AmpliTaq (Perkin–Elmer/Cetus), and either buffer A or buffer B (primers 32892 and 32894 with buffer A for leader peptide sequence/exon 1 and primers 31555 and 31557 with buffer B for exon 2). Buffer A contained 50 mM potassium chloride, 10 mM Tris·HCl (pH 8.3), 1.5 mM magnesium chloride, and 0.25% Nonidet P-40. Buffer B contained 10 mM Tris·HCl (pH 8.3), 50 mM potassium chloride, 3 mM magnesium chloride, and 0.01% Triton X-100. The mixtures were over-layered with light mineral oil and amplified for 30 cycles in a thermal cycler (Hybaid, Teddington, U.K.) as follows: for primer combination 32892/32894, 92°C (30 sec), 68°C (30 sec), and 70°C (2 min); and for primer combination 31555/31557, 92°C (30 sec), 60°C (30 sec), and 72°C (2 min).

Individual PCR products were labeled with ^{32}P as described (17). Briefly, 1-μl aliquots of PCR products were added to 25 μl of fresh PCR reaction mixture containing the appropriate buffer and primer pairs. Additionally, 50 μCi of [α-^{32}P]dCTP (specific activity, 3000 Ci/mmol; 1 Ci = 37 GBq; Amersham) were added. PCR amplification was then performed for 10 cycles, under the conditions described above, for each primer combination.

Single-Stranded Conformation Polymorphism (SSCP) Analysis. One-microliter samples of ^{32}P-labeled PCR products were added to 10 μl of SSCP loading buffer containing 15 mM EDTA, 0.05% SDS, and 50% (vol/vol) formamide. Samples were then denatured at 95°C for 5 min and super-cooled. Seven microliters was loaded on to a 50-cm-long nondenaturing SSCP gel as described (18). Analysis of the leader peptide sequence/exon 1 product was achieved on an 8% polyacrylamide gel containing 17.5% (vol/vol) glycerol and 1× TBE buffer (20) electrophoresed at 450 V at room temperature for 24 h. For exon 2 PCR products, a 5% polyacrylamide gel containing 10% glycerol electrophoresed at 400 V for 20 h at room temperature was used. After

Abbreviations: β_2M, β_2-microglobulin; SSCP, single-strand conformation polymorphism.

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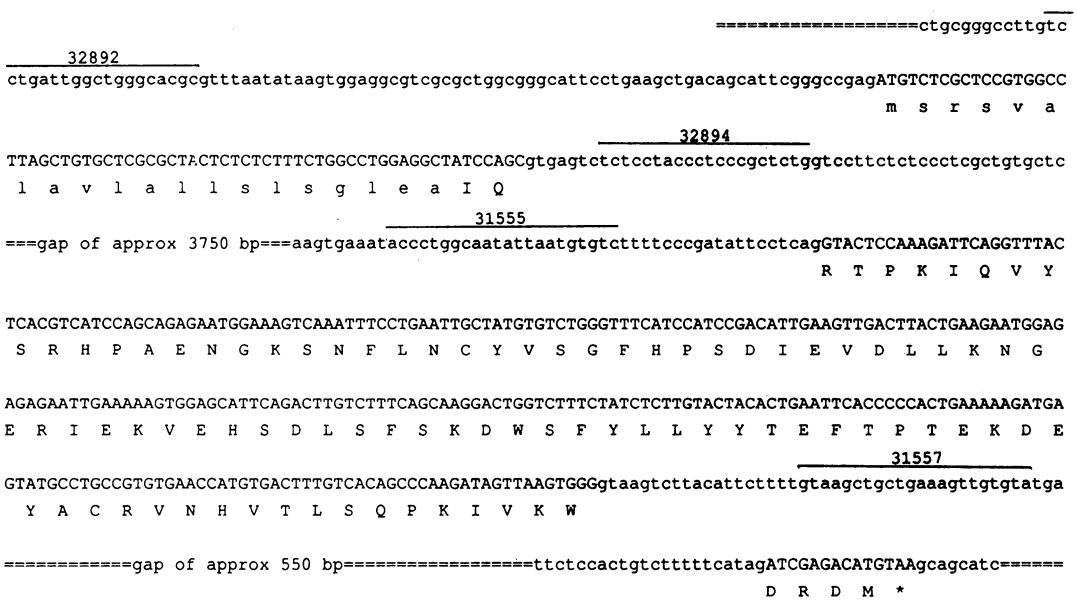

FIG. 1. β_2M genomic sequence. The locations of the primers used for PCR are indicated by lines above the sequence. Uppercase type is translated sequence. Lowercase type is untranslated sequence. The corresponding amino acids are shown below the nucleotide sequence. Data are taken from a published nucleotide sequence (16).

electrophoresis, the gels were dried and bands were located by autoradiography.

DNA Sequencing. DNA sequencing of PCR products was performed using the direct solid-phase sequencing system (19). Briefly, a PCR product for β_2M leader peptide sequence/exon 1 was amplified as described above with the biotinylated 3′ primer 32894. The PCR products were purified on a 2% low-melting-point agarose gel using TAE buffer (20) and visualized on a UV transilluminator. After removing the appropriate band, the DNA was recovered using a Mermaid kit (Bio 101) according to the manufacturer's protocol. Single-stranded DNA was then isolated using streptavidin-coupled Dynabeads M280 (Dynal, Oslo) as described (19). Single-stranded sequencing was performed using a Sequenase 2 kit (United States Biochemical) with the 5′ primer 32892 to prime the sequencing reaction. On completion of the reaction, the samples were heated to 85°C for 5 min then super-cooled. The immobilized template DNA was removed using a magnet (Dynal) and samples were electrophoresed on a standard 6% denaturing polyacrylamide gel, followed by gel drying and autoradiography. Sequencing in the opposite direction was achieved by using biotinylated 32892 primer in the PCR and the nonbiotinylated primer 32894 to prime the sequencing reaction. β_2M exon 2 was sequenced in a similar way using the appropriate combination of primers (see Fig. 1). The DNA was in this case, however, recovered from agarose gel purification using a Geneclean II kit (Bio 101).

β_2M ELISA. A β-galactosidase/anti-galactosidase ELISA method was used in this study (21). Monoclonal antibodies used were W6/32 (HLA class I–β_2M complex) (22), L368 (β_2M) (23), and BBM.1 (β_2M) (24).

RESULTS

Screening the β_2M Gene by SSCP. DNA was extracted from 37 established colorectal cell lines, 22 fresh tumor samples, and 22 normal controls and examined for mutations in the leader peptide sequence/exon 1 and exon 2 of the β_2M gene. Exons 1 and 2 were analyzed separately; PCR products were produced using the series of oligonucleotides and conditions described. Samples of these PCR products were labeled with ^{32}P then analyzed by the SSCP technique. One of the 22 fresh tumor samples examined by SSCP showed a bandshift in the leader peptide sequence/exon 1 PCR product (Fig. 2, sample 10). A band shift was also seen in the Burkitt lymphoma cell line, Daudi (Fig. 2, sample 2), which was used as a positive control. Daudi has been reported to have a G → C mutation in the initiator ATG sequence of the leader peptide (5). The 37 established cell lines examined were HCT-116, COLO 320DM, SW620, SW480, SW837, SW1222, SW48, DLD-1, T84, SW1417, CC20, HT-29, WiDr, HCT-15, CCO7, COLO 201, COLO 206, HCA46, HCA7, VACO4A, VACO10MS, HRA19, LS 174T, LS 411, LS 1034, LIM1863, SW403, LoVo, C10, C32, C70, C75, C80, C84, VACO5, VACO4S,

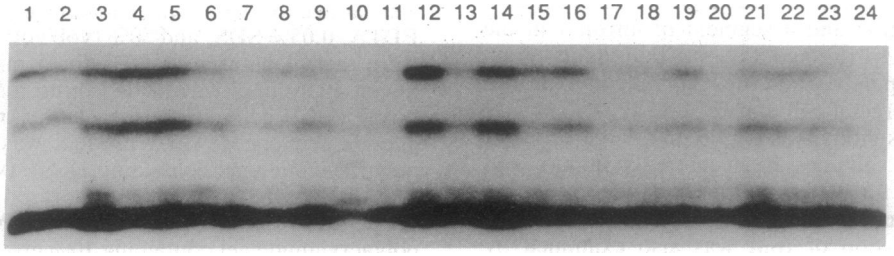

FIG. 2. Autoradiograph of SSCP analysis of β_2M leader peptide sequence/exon 1 PCR products from a series of fresh colorectal tumor samples. The products were amplified using the primers 32892 and 32894 (see Fig. 1) and labeled with ^{32}P. Samples were denatured and electrophoresed on a nondenaturing 8% polyacrylamide gel containing 17.5% glycerol. Lanes: 1, Bristol-8 (normal B cell line) wild-type control; 2, Daudi (Burkitt lymphoma cell line) containing a known mutation at the initiator ATG sequence; 3–24, a series of 22 fresh colorectal tumor samples.

Table 1. Mutations of β_2M identified by sequencing

Cell line	Exon	Homozygous/ heterozygous	Mutation	Stop codon location
LoVo	Leader sequence	Homozygous	CT deletion, codons 13–15*	Codon 36
HRA19	Leader sequence	Heterozygous	TCTT deletion, codons 14 and 15*	Codon 23
Sample 10 (fresh tumor)	Leader sequence	ND	CT deletion, codons 13–15*	Codon 36
SW48	Leader sequence	Heterozygous	CTCT deletion, codons 13–15*	Codon 23
	2	Heterozygous	Deletion, codon 47	Codon 82
C84	2	Heterozygous	G → A, codon 32	Asp → Asn
HCT-15/DLD-1	2	Heterozygous	C → A, codon 10	Mutation in codon 10
	2	Heterozygous	G → T, last base in intron 1	No stop codon
Daudi	Leader sequence	Homozygous	G → C, initiation codon (ATG)	Met → Ile

In the mutation column, * represent codons in the leader sequence. ND, not determined.

and JW. Three of these cell lines, (LoVo, HRA19, and SW48) demonstrated band shifts in their leader peptide sequence/exon 1 PCR product. All the 22 normal DNA samples had wild-type band patterns indicating, as expected, that no mutations were present.

SSCP analysis of exon 2 revealed band shifts in the cell lines SW48, DLD-1, HCT-15, and C84 (data not shown). None of the fresh colorectal tumor samples and the normal DNA samples demonstrated any differences from the wild-type pattern.

Sequencing of the Samples Positive by SSCP. All the samples demonstrating a band shift by SSCP were directly sequenced using PCR products.

The overall results are summarized in Table 1. The cell line LoVo is homozygous for a CT deletion in the leader peptide sequence as shown in Fig. 3. SW48 and HRA19 show different 4-bp deletions (CTCT for SW48 and TCTT for HRA19) in the same region of the leader peptide sequence. In addition, the presence of the wild-type sequence at these positions indicates that these cell lines are heterozygous for their respective mutations. SW48 has an additional heterozygous deletion of the adenosine in codon 47 in exon 2. Only one fresh colorectal tumor sample, sample 10, demonstrated a mutation—namely, a CT deletion in the leader peptide sequence in the same region as the cell line LoVo. As a control, the Burkitt lymphoma cell line, Daudi, was sequenced and shown to have a G → C mutation in the initiation ATG of the leader peptide sequence as reported elsewhere (5).

In exon 2, the colorectal cell line C84 has a heterozygous G → A point mutation in codon 32 (Table 1) changing an Asp to an Asn. Both the cell lines HCT-15 and DLD-1, which are derived from the same patient, demonstrate two mutations in exon 2 as shown in Fig. 4. A point mutation (G → A) was found at the last base of intron 1 and a C → A point mutation in codon 10 changes a Tyr into a stop codon.

Comparison of β_2M Mutations and Expression in Colorectal Cell Lines. The colorectal cell lines that were demonstrated by sequencing to have a mutant β_2M gene were examined for expression of β_2M by ELISA using a panel of antibodies. The cell lines SW48, HCT-15, and DLD-1 exhibit no reactivity with W6/32, which recognizes the β_2M–HLA-ABC complex, and the absence of free β_2M chains was confirmed in these cell lines by the lack of reactivity with the antibodies L368 and BBM.1 (Table 2). LoVo has previously been shown to be deficient in β_2M expression (25). HRA19 and C84 are both heterozygous for single β_2M mutations and show intermediate levels of β_2M expression with antibodies W6/32 and BBM.1. However, the reactivity of L368 to the cell line C84 appears to be normal. The colorectal cell line HT-29, which has a normal β_2M gene, is included in Table 2 as a positive control.

DISCUSSION

We have screened a large panel of fresh colorectal tumor samples and established colorectal cell lines for β_2M changes,

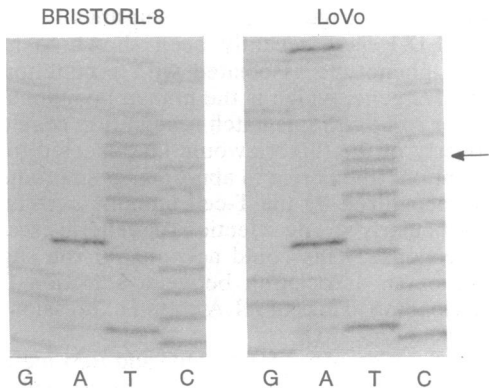

FIG. 3. Autoradiograph from a sequencing gel of β_2M leader peptide sequence/exon 1 PCR products. Samples were Bristol-8, a normal B-cell line showing the wild-type sequence, and LoVo, a colorectal cell line showing a homozygous CT deletion in the 8-bp CT repeat sequence. The deletion is indicated by an arrow.

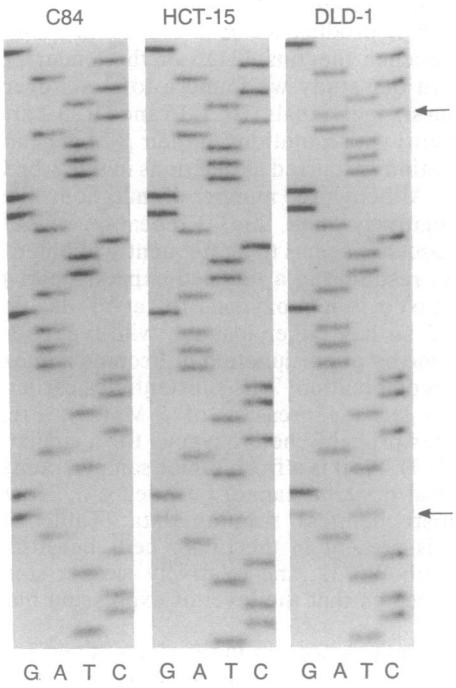

FIG. 4. Autoradiograph from a sequencing gel of β_2M exon 2 PCR products. Samples were C84, a colorectal cell line showing the wild-type sequence in this region of exon 2; HCT-15 and DLD-1, two colorectal cell lines (derived from the same patient) that show the same two heterozygous mutations. The upper arrow indicates the site of a C → A mutation and a G → T mutation is indicated by the lower arrow.

Table 2. Comparison of β_2M mutations and expression of β_2M determined by ELISA

Cell line	Mutation location	Homozygous/ heterozygous	ELISA		
			W6/32	L368	BBM.1
HT-29	None		3389	Over	2857
LoVo	Leader sequence	Homozygous	D	D	D
HRA19	Leader sequence	Heterozygous	923	2865	305
SW48	Leader sequence and exon 2	Both heterozygous	141	184	492
C84	Exon 2	Heterozygous	1588	Over	1795
HCT-15	Two mutations in exon 2	Both heterozygous	0	0	370
DLD-1	Two mutations in exon 2	Both heterozygous	75	92	234
Sample 10 (fresh tumor)	Leader sequence	ND	Positive		Moderately positive

D, previously shown to be deficient for β_2M (25); ND, not determined. Results for sample 10 are from immunohistochemical staining (L. Kaklamanis, personal communication). Over (in the ELISA), positive value that exceeded instrument range.

initially by a quick screening technique using SSCP, followed by sequencing those samples that indicated band shifts by SSCP. We examined 22 fresh tumors and found 1 mutation (4.5%) and examined 37 established cell lines that were derived from 33 individuals (HCT-15/DLD-1, WiDr/HT-29, COLO 201/COLO 206, SW480/SW620 are pairs of lines derived from the same patients) and found 5 with mutations (15.2%). We found no evidence of mutations by SSCP in 22 normal DNA samples. In a recent review of the SSCP technique, it was claimed that in ideal conditions and using DNA fragments of ≈300 bp or smaller, an efficiency of between 95 and 100% should be achieved (26). In our system most of the β_2M gene was investigated. The first two codons of β_2M in exon 1, the leader peptide sequence, and the sequence up to 6 bp upstream of the TATA box were included in the 179-bp PCR product, and the whole of exon 2 was included in a 361-bp PCR product using intronic sequences for PCR primers. These two sequence fragments, therefore, include 96% of the translated codons of the mature β_2M molecule.

The mutations found in the leader peptide sequence/exon 1 179-bp PCR product all occurred in the same 8-bp CT repeat sequence (codons 13–15 of the leader peptide sequence). In our study we found a total of seven samples taken from six individuals (HCT-15 and DLD-1 are from the same patient) with mutations in their β_2M gene and four of these mutations occurred as deletions in this 8-bp CT repeat sequence. Although the number of mutations found in this study is relatively small, this CT repeat region of the leader peptide sequence seems to be frequently mutated in at least one allele, resulting in a reduced expression of β_2M. The cell line LoVo is homozygous for a CT deletion in this region and so has either lost the wild-type β_2M bearing chromosome by nondisjunction or become homozygous by somatic recombination. This substantiates earlier evidence that LoVo lacks expression of β_2M (25). Fresh tumor sample 10 is probably heterozygous for a similar CT deletion (Table 1). As it is a fresh tumor sample, contamination from normal material cannot be ruled out. However, the immunohistochemical staining data (Table 2) suggest that β_2M is present in the tumor cells because they are positive with W6/32, and relatively weaker staining with BBM.1 indicates that the level of expression may well be reduced.

All the deletions seen in the leader sequence cause stop codons further down the sequence (see Table 1). However, as these mutations occur in the leader sequence and result in frame shifts, it is probable that no β_2M is translated from affected alleles.

The mutations seen in exon 2 are predominantly point mutations. The C → A mutation in codon 10 of the cell line HCT-15 (Fig. 4) has been reported (7). The same mutation is present in the DLD-1 cell line derived from the same patient. In the same report (7), a clone of HCT-15 was shown to have the first 11 bp of exon 2 deleted. Our data suggest that in the lines of HCT-15 and DLD-1 that we analyzed, there is a point mutation (G → T) at the last base in intron 1 (Fig. 4). This G → T mutation alters the GT and AG splice site, reported to be crucial for splicing to occur (27), to GT and AT, respectively.

The expression of β_2M as determined by ELISA reflects the events that have occurred at the gene level. Where there is a single allele affected, the expression is reduced as seen in C84 and HRA19. In the case of LoVo, which is homozygous for a deletion in the leader sequence, the β_2M expression is negative (25). Cell lines SW48, DLD-1, and HCT-15 have two distinct mutational events and in these cases the β_2M expression is also negative, which indicates that in each cell line both alleles are affected.

These data provide the most direct evidence so far for the effect of selection to escape T-cell immune response on β_2M expression. The frequency of the CT deletions in the leader region is intriguing and suggests an interaction between mutation and selection—namely, that it is the most frequent mutation giving the requisite effect that is most likely to be selected for and so widely expressed in a tumor.

The homozygosity of LoVo for a β_2M mutation is interesting in that it indicates the expected two-step process to achieve absence of β_2M expression—namely, first selection for an inactivating mutation and then for loss of the remaining wild-type β_2M allele. There must be some advantage to the first step, due perhaps simply to reduced surface expression of β_2M and so HLA class I molecules, connected with some increase in resistance to T-cell killing. This heterozygous effect is seen more directly in C84 and HRA19, which have single β_2M mutations and reduced β_2M expression. It is intriguing that the cell lines LoVo, HCT-15, and DLD-1 have recently been shown to have the "mutator" phenotype associated with a mutation in the human *MSH2* gene, which is the human homologue of the *Escherichia coli mutS* mismatch recognition gene (28–31). This property of the tumors would be expected to lead to many mutations giving rise to abnormal gene products that could be recognized by the T-cell immune system and so lead to particularly strong selection for general escape from immune response. This could account for the suggestive indication of an association between selection for β_2M mutations leading to loss of HLA class I expression and the mutator phenotype (31).

The sole function of the HLA-ABC and β_2M molecules is in the immune system, and so our data clearly substantiate the role of immune response to colorectal tumors, at least, in leading to selection for mutations that allow cancers to escape from immune T-cell attack.

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1. Townsend, A., Ohlen, C., Bastin, J., Ljunggren, H.-G., Foster, L. & Karre, K. (1989) *Nature (London)* **340,** 443–448.
2. Smith, M., Bodmer, W. & Bodmer, J. (1988) *Lancet* **i,** 823–824.
3. Csiba, A., Whitewell, H. L. & Moore, M. (1984) *Br. J. Cancer* **50,** 699–709.
4. Kaklamanis, L. & Hill, A. (1992) *Cancer Surv.* **13,** 155–171.
5. Rosa, F., Berissi, H., Weissenbach, J., Maroteaux, L., Fellous, M. & Revel, M. (1983) *EMBO J.* **2,** 239–243.
6. D'Urso, C. M., Wang, Z., Cao, Y., Tatake, R., Zeff, R. A. & Ferrone, S. (1991) *J. Clin. Invest.* **87,** 284–292.
7. Gattoni-Celli, S., Kirsch, K., Timpane, R. & Isselbacher, K. J. (1992) *Cancer Res.* **52,** 1201–1204.
8. Browning, M. J., Krausa, P., Rowan, A., Bicknell, D. C., Bodmer, J. G. & Bodmer, W. F. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 2842–2845.
9. Brattain, M. G., Fine, W. B., Mahnaz Khalaed, F., Thompson, J. & Brattain, D. E. (1981) *Cancer Res.* **41,** 1751–1756.
10. Quinn, L. A., Moore, G. E., Morgan, R. T. & Woods, L. K. (1979) *Cancer Res.* **39,** 4914–4924.
11. Leibovitz, A., Stinson, J. C., McCombs, W. B., McCoy, C. E., Mazur, K. C. & Mabry, N. D. (1976) *Cancer Res.* **36,** 4562–4569.
12. McBain, J. A., Weese, J. L., Meisner, L. F., Wolberg, W. M. & Willson, K. V. (1984) *Cancer Res.* **44,** 5813–5821.
13. Kirkland, S. C. & Bailer, I. G. (1986) *Br. J. Cancer* **53,** 779–785.
14. Klein, E., Klien, G., Nadkarni, J. S., Nadkarni, J. J., Wigzell, H. & Cliffor, P. (1968) *Cancer Res.* **28,** 1310–1330.
15. Degli-Eposti, M. A., Griffiths, S. J., Daly, L. N., Witt, C. S., Simons, M., Carcassi, C., Albert, E. D., Giphart, M. J. & Dawkins, R. L. (1993) *Hum. Immunol.* **38,** 3–16.
16. Gussow, D., Rein, R., Ginjaar, I., Hochstenbach, F., Seemann, G., Kottman, A. & Ploegh, H. L. (1987) *J. Immunol.* **139,** 3132–3138.
17. Bicknell, D. C., Markie, D., Spurr, N. K. & Bodmer, W. F. (1991) *Genomics* **10,** 186–192.
18. Orita, M., Suzuki, Y., Sekiya, T. & Hayashi, K. (1989) *Genomics* **5,** 874–879.
19. Hultman, T., Murby, M., Stahl, S., Hornes, E. & Uhlen, M. (1990) *Nucleic Acids Res.* **18,** 5107–5112.
20. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
21. Durbin, H. & Bodmer, W. F. (1987) *J. Immunol. Methods* **97,** 19–27.
22. Barnstable, C. J., Bodmer, W. F., Brown, G., Galfre, G., Milstein, C., Williams, A. F. & Ziegler, A. (1978) *Cell* **14,** 9–20.
23. Lampson, L. A., Fisher, C. A. & Whelan, J. P. (1983) *J. Immunol.* **130,** 2471–2478.
24. Brodsky, F. M., Bodmer, W. F. & Parham, P. (1979) *Eur. J. Immunol.* **9,** 536–545.
25. Brodsky, F. M., Parham, P., Barnstable, C. J., Crumpton, M. J. & Bodmer, W. F. (1979) *Immunol. Rev.* **47,** 3–61.
26. Hayashi, K. & Yandell, D. W. (1993) *Hum. Mutat.* **2,** 338–346.
27. Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. & Chambon, P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4853–4857.
28. Leach, F. S., Nicolaides, N. C., Papadopoulos, N., Liu, B., Jen, J. *et al.* (1993) *Cell* **75,** 1215–1225.
29. Fishel, R., Lescoe, M. K., Rao, M. R. S., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M. & Kolodner, R. (1993) *Cell* **75,** 1027–1038.
30. Shibata, D. (1994) *Nature Genet.* **6,** 273–281.
31. Bodmer, W. F., Bishop, T. & Karran, P. (1994) *Nature Genet.* **6,** 217–219.