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It has been difficult to genetically map the genes encoding tumor Ags because they arise as a consequence of somatic mutational events. CTL-mediated immunoselection can impose potent immune selective pressure against tumor cells, resulting in the survival of rare tumor Ag-loss variants. We subjected a heterozygous 3-methylcholanthrene-induced murine sarcoma cell line to CTL immunoselection, selecting for the loss of a tumor-specific Ag, recognized antigen from MCA-induced tumor 1 (Ram1). Several variants eluded CTL recognition by genetic loss of the hemizygously expressed tumor-specific Ag epitope. A frequently observed genetic escape mechanism was spontaneous mitotic recombination resulting in loss of heterozygosity on chromosome 4. Higher density genetic analyses along with functional confirmation with an independently produced chromosome 4 loss of heterozygosity variant positioned the Ram1 locus to a distal 7.1 cM interval on chromosome 4. This region of the mouse genome is rich in tumor-modifier genes and this positioning of Ram1 may thus provide insight into the genetic basis of 3-methylcholanthrene-induced tumor Ags. *The Journal of Immunology*, 2001, 167: 5143–5149.

The CD8⁺ CTL is a vital component of the immune system’s response to invasive agents, non-self cells, and tumors. In the case of tumors, CTLs recognize, through their TCR, tumor Ags in the context of appropriate class I MHC molecules. These Ags are short, processed peptides derived from aberrant or improperly regulated cellular proteins, and are presented on the cell surface for evaluation by CTLs. If the CTL recognizes the peptide Ag as foreign, it kills the target cell either by perforin-mediated lysis or by inducing apoptosis. There is considerable importance to understanding the molecular etiology of tumor Ags that can serve as targets for CTL-mediated immunotherapy. Toward this end, two methodologies have proven to be most fruitful: functional screening of transfected genomic or cDNA libraries and biochemical analysis of peptides eluted from tumor cells. The use of CTLs to screen tumor-specific genomic or cDNA expression libraries to isolate genes encoding tumor Ags has resulted in the characterization of a variety of CTL-recognized tumor Ags (1–3). Edman degradation and mass spectrometric analysis of the eluted peptide fractions from tumor cells have led to the molecular identification of additional tumor Ags (4, 5).

The above methodologies are genome-wide in that they screen for “hits” from all expressed genes or from peptides encoded by any gene in the genome. On the other hand, the ability to efficiently establish the chromosomal region associated with peptide ligands recognized by CTLs could accelerate the identification of tumor Ags because it greatly reduces the number of candidate genes. Efficient establishment of chromosomal position of tumor Ag genes promises to be even more valuable with the completion of the human and mouse genomes.

Somatic cell selection in vitro has proven to be a powerful genetic tool. When a transformed cell line is subjected to strong negative selection pressure against a genetically determined trait, rare mutant clones survive. If the negatively selected cell line is heterozygous for the locus conferring the trait, a frequently observed mutational event is a loss of heterozygosity (LOH) of the locus along with adjoining chromosomal material (6–8). The extent of this LOH, which can be determined with molecular markers, identifies the chromosomal position of the gene encoding the trait. CTL lines have proven to be remarkably efficient in selecting for Ag-loss variant cells in vitro because they are proficient in lysing target cells (7–13). In fact, LOH arising as a consequence of CTL immunoselection has facilitated the high resolution mapping of several non-H2 histocompatibility Ags (7, 8, 12, 14, 15).

In principle, this same approach could be extended to map genes encoding tumor Ags. CTLs specific for a unique tumor-specific Ag (TSA) can be readily raised against 3-methylcholanthrene (MCA)-induced sarcomas (13, 16–18). Such CTLs have been shown to select in vitro for stable TSA-loss variants (13). Therefore, we hypothesized that it should be possible to detect LOH after immunoselection of a MCA-induced tumor derived from a heterozygous mouse, and that these regions of LOH could be used to map the TSA-encoding locus. We show here that CTL immunoselection followed by LOH analysis of the escape variants is an efficient method to genetically map a dominant MCA-induced TSA. Analysis of the escape variant’s LOH footprint mapped the locus, recognized antigen from MCA-induced tumor 1 (Ram1), controlling the antigenic epitope to a 7.1 cM region on chromosome 4. This region is known to harbor several tumor susceptibility/suppressor genes and frequently displays LOH in both spontaneous and induced tumors.

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5 Abbreviations used in this paper: LOH, loss of heterozygosity; MCA, 3-methylcholanthrene; TSA, tumor-specific Ag; CML, cell-mediated lysis; GIA, growth inhibition assay; Ram1, recognized Ag from MCA-induced tumor 1.
Materials and Methods

Cell lines

Generation of the MCA-induced fibrosarcoma bs15.1 was described previously (19). The myoblast cell line F1myo was established from muscle of a nonchemically treated neonatal (C57BL/6J × SPRET/EiF1) mouse as described elsewhere (19). The bs15.1-derived cell line 15A1 is a Ram1-loss cell line produced from a previous immunoselection experiment (13). The Ram1-specific cloned BxS/15.4 CTL line used for immunoselection recognizes a unique Kb-restricted Ag on the bs15.1 tumor (13). The allo-anti-H2* CTL line SPM4 has been described previously (13). The CTL line BxS/3E1 specific for a C57BL/6 (B6) minor H Ag encoded on the distal end of chromosome 4 was derived from spleen cells from B6.Spr4 mice primed and restimulated repeatedly in vitro with B6 spleen cells. B6.Spr4 mice, kindly provided by Dr. E. Eicher of The Jackson Laboratory, carry a SPRET/Ei congenic segment from the distal portion of chromosome 4. The BxS/3E1 CTL line is specific for a B6-derived chromosome 4 minor H Ag as evidenced by its ability to lyse B6-derived target cells but not B6.Spr4-derived target cells (data not shown).

Immunoselection

The immunoselection procedure is a modification of that described elsewhere (7, 8, 13). One hundred bs15.1 tumor cells were aliquoted into 96-well flat-bottom microcell plates (Corning, Corning, NY) and cultured in 10% FBS-supplemented DMEM at 37°C in a 5% CO2 humidified atmosphere. The bs15.1 cells were allowed to expand for 3 days to reach 10^5 cells/microcell to maximize the probability that variants detected arose from independent mutational events. The cells were then trypsinized and added to microwells already seeded with 1 × 10^5 BxS/15.4 CTLs in a total of 200 µl of culture medium supplemented with 100 U/ml IL-2. After 7–10 days of culture, the rare “growth-positive” microwells were detected. The cells in these microwells, which presumably represented rare Ag-loss variant bs15.1 tumor cells, were reimmunoselected with BxS/15.4 CTLs, and growth-positive microwells were expanded for functional and molecular tests. To produce the B6 minor H Ag-loss chromosome 4 LOH variant bs/E2, similar methods were used with the exception that the CTL line BxS/3E1 was used for immunoselection.

Growth inhibition assay (GIA)

To screen for potential TSA-loss variants, a modification of the GIA was used (7, 8). This assay exploits the fact that the proliferation of transformed cell lines is suppressed by the specific lytic activity of CTLs. Thus, the inhibition of tumor cell growth indicates that the transformed cell expresses the cognate Ag against which the CTLs are directed. Nonimmunoselected bs15.1 cells or growth-positive microwells that arose after CTL immunoselection were disaggregated with trypsin and added in a volume of 100 µl of 5% FBS-supplemented DMEM to round-bottom microwells (Corning) in concentrations of 1–3 × 10^4 tumor cells. For effectors, 0.5–1 × 10^5 of the specified CTLs were harvested from passage 4–5 days after subculture, washed twice to eliminate residual IL-2, and added in 100 µl of medium to the tumor-cell-containing microwells. Under such conditions, the CTLs incorporate negligible amounts of [3H]thymidine. After 24–36 h of coculture, 1 µCi/well of [3H]thymidine was added and after 8–12 h, mean [3H]thymidine incorporation of triplicate cultures was determined. Results are presented as percent growth inhibition, calculated as the ratio of CTL-inhibited [3H]thymidine incorporation to [3H]thymidine incorporation without CTLs.

Flow cytometric analysis

FACS was performed using a FACSscan (BD Biosciences, Mountain View, CA). Propidium iodide gating was used to exclude dead cells and the relative fluorescence of fluorescein- or rhodamine-conjugated mAbs was measured. mAbs specific to K4 (31-3-4), K8 (28-13-3), and D8 (28-14-8) were used; none of which cross-react with H2 alloantigens from SPRET/Ei. Alternately, biotinylated Abs were used and detected with PE-conjugated streptavidin (Molecular Probes, Eugene, OR).

Cell-mediated lysis (CML) assay

CML assays were performed and percent specific cytotoxicity was determined following established procedures (20).

Screening of variant DNA with microsatellite markers

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Screening of variant DNA with microsatellite markers

DNA was isolated from the bs15.1-derived tumors as described previously (19). For genotypic analysis, primers for MIT microsatellite markers showing a discernible (≥10% difference) PCR polymorphism between B6 and the inbred Mus spretus strain SPRET/Ei were obtained from Research Ge-

netics (Huntsville, AL) and The Jackson Laboratory Microchemistry Service. MIT markers mapping near the telomere of each of the 19 autosomal chromosomes were analyzed: D1 Mit150, D2 Mit657, D3 Mit18, D4 Mit68, D5 Mit102, D6 Mit201, D7 Mit12, D8 Mit19, D9 Mit151, D10 Mit180, D11 Mit214, D12 Mit65, D13 Mit35, D14 Mit131, D15 Mit34, D16 Mit86, D17 Mit123, D18 Mit3, and D19 Mit108. Ten variants that had escaped immunoselection by losing cell surface MHC class I were also screened with the chromosome 4 single-stranded conformational polymorphism (SSCP) marker, Cdc42 (see below), and D17 Mit123. Marker positions, primer sequences, and strain polymorphisms were obtained from The Jackson Laboratory’s Mouse Genome Informatics SSLDB database (www.informatics.jax.org). For pooled screening, equivalent amounts of DNA from 14 Ag-loss variants were combined to produce two pools (A and B), each with a DNA concentration of ~50 ng/µl. Parental bs15.1 DNA with a concentration of 50 ng/µl was also used with every primer pair tested. PCR conditions consisted of an initial 95°C denaturation for 3 min; 40 cycles of 94°C for 30 s, 72°C for 30 s; and 72°C final extension for 10 min followed by a soak at 4°C. Different annealing temperatures, Tanneal from 50 to 60°C, were used in the program with various primer pairs to accommodate the different Ts of the MIT marker primers. The PCR products were subjected to electrophoresis through 3% NuSieve (FMC BioProducts, Rockland, ME), 1% agarose gels, and visualized with a UV transilluminator following staining with ethidium bromide.

Variant analysis by SSCP

LOH screening was also performed by SSCP using gene-specific primers. Primers were designed for the chromosome 4 genes Paftr, Fgr, Cappb1, Cdc42, Pla2g2c, and Pax7 based on sequences deposited at Mouse Genome Informatics. Primer sequences were as follows: Paftr-F, 5‘-TGGTATACCCGCTTCTTCCGA-3‘, Paftr-R, 5‘-GTCAGCACTATGAGTACCCCAAG-3‘; Fgr-F, 5‘-CAGGGTATGACCAACTGCAACAACCGGGAAGG-3‘, Fgr-R, 5‘-TGAGGTTGAATAAGTCTTTCCA-3‘; Cappb1-F, 5‘-ACGGCCATGCTACGTGTTAGGTC-3‘, Cappb1-R, 5‘-AGCTATGCCCTTTC-3‘; Cdc42-F, 5‘-GGTTGGTGGTGTGTGATGTTGTC-3‘, Cdc42-R, 5‘-TGTCCTGTTGATCTAGGGTGTC3‘; Pla2g2c-F, 5‘-ACCCCTCGCATGACT-3‘, Pla2g2c-R, 5‘-CCTGCTCCTTGAATGCCTGTTT-3‘; Pax7-F, 5‘-TCCAGAGGAGTTGACCCTAT-3‘, and Pax7-R, 5‘-CCTAGAGGACTCGTACCCTGTA-3‘. PCR products using these primer pairs were performed above using an 80°C annealing temperature. PCR products were mixed with an equal volume of SSCP loading buffer (75% v/v dimethylformamide, 12.5% v/v 100 mM NaOH, 12.5% v/v blue dextran), heated at 95°C for 2 min, and rapidly plunged in ice. The samples were subjected to electrophoresis through a 0.5% MDE polyacrylamide gel at 4°C (FMC BioProducts, Rockland, ME) and visualized by silver staining using SILVER SEQUENCE staining reagents following the manufacturer’s protocol (Promega, Madison, WI). Photographs of the gels were captured using a light box and Automatic Processor Compatible paper from Promega. Heterozygosity or LOH determinations were made by comparing variant SSCP bands with those of the parental tumor bs15.1, B6 and SPRET/Ei.

Statistical analysis of LOH variants

The probability of detecting chromosome 4 LOH variants was determined by the Poisson binomial. The probability of detecting at least one variant with LOH at Cdc42 among the MHC-loss variants was p = 0.41 (probability of detecting LOH), q = 0.59 (probability of not detecting LOH) and n = 10: P (one or more H2-loss variants with LOH) = 1 – P (no variants with LOH); 1 = 1 – (pC0)((q)n); 1 = 1 – (pC0)(0.41)(0.59)n; 0.995 or 99.5%.

Results

Immunoselection and characterization of bs15.1 Ag-loss variants

The tumor clone bs15.1 was derived from a MCA-induced tumor arising in a male (C57BL/6J × SPRET/EiF1) mouse. Immunization of these mice with bs15.1 led to the development of a CTL clone, BxS/15.4, that recognized a Kβ-restricted dominant epitope, referred to as Ram1, expressed on bs15.1 but not by other MCA-induced (B6 × SPRET/EiF1) tumors (19). To generate clones due to LOH of the gene controlling the Ram1 epitope, we subjected microcultures of the bs15.1 tumor to immunoselection by BxS/15.4 CTLs. Although the CTLs killed all viable tumor cells from most seeded microwells, 50 microwells were selected that had observable tumor cell growth by 7–10 days. These growth-positive microwells were then screened for Ram1 Ag expression using a GIA (11, 12). Fig. 1A shows data from one such screening assay.
As expected, BxS/15.4 CTLs inhibited the proliferation of the parental bs15.1 tumor cells; however, the CTLs failed to inhibit the proliferation of a Ram1-negative (C57BL/6J/H11003SPRET/Ei)F1-derived myoblast cell line or of the immunoselected tumor cell cultures. These results suggested that the immunoselection procedure gave rise to stable Ag-loss variant cells. Either a mutational loss of H2-Kb or of the gene encoding Ram1 could allow survival of the bs15.1 tumor cells. To determine whether any of the variant cell lines escaped immunoselection due to MHC loss, we screened these microcultures by FACS analysis using H2-Kb and H2-Dd-specific mAbs. As summarized in Fig. 1B and Table I, of the 50 growth-positive wells, 11 cultures failed to express detectable H2-Kb/Dd alloantigens and 29 continued to express Kb/Dd. (The remaining 10 were a mixture of H-Kb/Dd-positive and -negative tumor cells, indicating that the cultures had heterogeneous populations including some that were Kb/Dd- and Kb/Dd+; these likely represent multiple mutational events.)

To confirm the GIA Ag typing, we performed conventional CML assays on a subset of the variant cell lines. Representative data in Fig. 1C show that the immunoselected variant cell lines that were either H2-Kb positive or H2-Dd negative were completely resistant to lysis by anti-Ram1-specific BxS/15.4 CTLs, while the parental bs15.1 tumor was efficiently lysed (left panel). However, only those cell lines that demonstrated serological expression of H2b were lysed by allo-H2b CTLs (Fig. 1C, right panel). The combined results provided functional evidence for two classes of mutational events that led to escape from immunoselection: loss of the H2-Kb alloantigen and specific loss of the Ram1 peptide epitope.

**Genome-wide marker scan identifies LOH on chromosome 4**

In addition to H2 loss, variants could escape immunoselection by mutational loss of the gene controlling Ram1 due to LOH of the Ram1 gene along with flanking loci. Since the most commonly observed LOH is a single mitotic recombinational event starting proximal to the selected locus and extending to the telomere (6, 8, 21, 22), we surveyed the variants using markers mapping to the distal segments of each of the 19 autosomal chromosomes. Since analysis of pooled DNA samples has proven to be an efficient way to screen for a common LOH (8, 23), we screened the variant DNAs in pools. Using this analysis, an allele-specific band weakening (as compared with the parental tumor line) was observed at telomeric markers on chromosomes 4, 5, and 11 (data not shown).

To test chromosome linkage more directly, we analyzed DNAs from each of the 29 Ram1-negative variant cell lines. When individual variants were tested with D5Mit102 and D11Mit214, we failed to confirm a LOH, thereby suggesting that the apparent band weakening (as compared with the parental tumor line) was observed at telomeric markers on chromosomes 4, 5, and 11 (data not shown).

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is shown in Fig. 3. Thirteen of the variants demonstrated LOH at distal chromosome 4. Six variants (2B9, 3C3, 4A1, 4C7, 1F3, and 15/H90041) demonstrated a LOH consistent with a single mitotic recombinational event, while 3 (1G10, 2D7, and 2D11) demonstrated an interstitial LOH. Variants 2D10 and 4D4 demonstrated LOH extending to the end of the chromosome with the exception of reproducible heterozygosity at a single gene, Fgr, within the boundaries of the LOH (Fig. 3A). However, variants 1C7 and 4G10 were excluded from further analysis because they demonstrated B6 allele-specific band weakening at several different markers (Fig. 2), which is consistent with nonclonality of the variant cultures. The resulting LOH map suggested a location for Ram1 (Fig. 3A). Two variants, 2B10 and 4D4, localized the proximal end of Ram1 just distal to D4 Mit204. Variant 2D11 tested homozygous at Pax7 and heterozygous for the tightly linked D4 Mit233 marker, thus fixing the distal boundary between these two loci at 69.0 cM. Taken together, the results are consistent with Ram1 mapping to a 7.1 cM interval between 61.9 and 69.0 cM on chromosome 4. Along with the parental bs15.1 cell line, the remaining 16 variants did not show LOH at any of the markers tested, indicating that these variants failed to express Ram1 as the result of an undefined mutational event.

**Statistical analysis of MHC class I-loss variants supports mapping of Ram1 to chromosome 4**

It is conceivable that the above described chromosome 4 LOHs could have arisen through processes unrelated to the presence of the Ram1 locus on this chromosome. Chromosome 4 LOH could occur as a consequence of random chromosomal instability or Ag-independent selection during tumor progression. If this were the case, one would expect to detect chromosome 4 LOH variants among the H2-loss variants, as they were treated identically to the specific Ram1 Ag-loss variants. To address this possibility, we typed 10 H2b-loss variants for the chromosome 4 Cdc42 gene and all proved heterozygous for this gene (Fig. 4). In contrast and consistent with LOH of the MHC as a common mechanism giving rise to tumor escape, nine of these variants showed a LOH at the chromosome 17 marker D17 Mit123 (Fig. 4). In our preceding analysis of the H2b-positive variants (Fig. 3), 11 of the 27 unambiguous variants showed a LOH at Cdc42. If the LOH arose as a consequence of events unrelated to anti-Ram1 immunoselection and given that 11 of 27 of the H2b-positive variants displayed a chromosome 4 LOH for Cdc42, Poisson statistics predict that there is less than a 1% binomial probability that all 10 of the H2-loss variants analyzed would remain heterozygous for this chromosome 4 marker. In other words, if LOH at Cdc42 was simply a random event, there is a >99% probability that at least one LOH at Cdc42 would have been detected among the MHC-loss variants.

**Independent derivation of a chromosome 4-loss variant confirms Ram1 mapping**

Given that the association between Ram1 and chromosome 4 relied heavily on statistical probability, we tested more directly whether the Ram1 Ag is controlled by a B6-encoded locus on chromosome 4. To do so, we produced a bs15.1 variant cell line...
with chromosome 4 LOH, generated by an immunoselection scheme independent of Ram1 expression. To do so, we took advantage of a B6-encoded minor H Ag that mapped to the distal end of chromosome 4 (see Materials and Methods). Using a CTL line specifically reactive against this minor H Ag (BxS/3E1), we immunoselected the bs15.1 tumor cell line. The variant cell line arising from this round of CTL immunoselection, bs/E2, demonstrated a LOH starting distal to D4 Mit204 and included several chromosome 4 loci (Fig. 3B). After verifying H2b expression, the bs/E2 and parental bs15.1 cell lines were tested by a CML assay for expression of the Ram1 Ag (Fig. 5). Although bs15.1 was lysed by Ram1-specific BxS/15.4 CTLs along with minor H Ag-specific BxS/3E1 CTLs and allo-H2b-specific S/4 CTLs, bs/E2 was lysed only by the allo-specific S/4 CTLs. The fact that bs/E2 variant cells were negative for Ram1 confirms the mapping of this TSA to the

![Figure 3](image1.png)

**FIGURE 3.** Summary of chromosome 4 LOH analysis of Ram1 escape variants. **A,** The LOH patterns of variants that display loss of the B6 allele for the indicated markers tested are shown. Marker positions are given in centiMorgan units and were obtained from Mouse Genome Informatics (www.informatics.jax.org). Regions across which B6 allele loss was observed are depicted as □. Areas of unknown/untested LOH. Variants whose LOH map defines the boundaries of Ram1 are highlighted in bold. *, 2D11 tested homozygous for Pax7 (Fig. 2, third panel) but tested heterozygous for the tightly linked marker D4 Mit233. **B,** The LOH pattern of minor H Ag-specific immunoselected variant bs/E2.

![Figure 4](image2.png)

**FIGURE 4.** Genetic analysis of MHC-loss variants. Top panel, Analysis of MHC-loss variants at the chromosome 4 marker Cdc42. Bottom panel, Analysis of the same MHC-loss variants at the chromosome 17 marker D17 Mit123. Parental tumor (BS) and homozygous parents (B, S) banding patterns are indicated.

![Figure 5](image3.png)

**FIGURE 5.** Functional characterization of variant bs/E2. Both bs15.1 and bs/E2 express H2b but bs/E2 fails to express Ram1. bs15.1 (A) and bs/E2 target cells (B) were tested in a CML assay using BxS/15.4 (anti-Ram1), BxS/3E1 (anti-minor H Ag on B6 chromosome 4), and S/4 (anti-allo-H2b) CTLs as effector cells.
indicated site on chromosome 4. In addition, the chromosome 4 breakpoint observed for bs/E2 corroborates the proximal boundary of the Ram1 locus (Fig. 3B).

Discussion
Forward genetic approaches, i.e., approaches that exploit chromosomal mapping of a biological trait as part of the gene discovery process, are fundamental tools of eukaryotic genetics. However, it has been difficult to apply such approaches to the genetics of tumor Ags because these Ags arise by somatic mutational events. It has been known for many years that CTLs directed against tumor Ags in vivo and in vitro can impose powerful immunoselective pressure, resulting in tumor cell escape variants that display a loss of either MHC alloantigens or the tumor Ags themselves (9, 13, 24–29). In this study, we demonstrate that CTL immunoselection in vitro is a reliable and efficient method to map a tumor Ag gene.

The key components are an in vitro-adapted tumor line from a heterozygous host and a CTL line that recognizes a neo-Ag on the tumor cells. For human tumors, sufficient heterozygosity for LOH analysis is commonplace. In our studies of CTL-immunoselected MCA tumors described here and in alloantigen-encoding gene mapping studies using CTLs to select Abelson virus-transformed lymphoblast tumors, chromosomal instability during in vitro culture has not confounded LOH analysis (8). Although this approach is based on selection in vitro, both MHC-loss and specific tumor Ag-loss escape variants are also known to arise in vivo (27, 29–31). In principle, LOH analysis on panels of human tumors could identify shared tumor Ag genes whose loss permits immune escape in vivo.

MCA-induced tumors provide a valuable and controlled model system to understand the relationship between mutations associated with chemical carcinogenesis and the TSAs against which the immune response can be directed. However, the gene targets for these mutations remain poorly understood. In this regard, the map position of Ram1 is intriguing. The region of LOH covering the Ram1 gene locus is dense in oncogenes and suppressor genes including Tslr2, Tslr3, Fgr, Cdc42, Ssic1, Sluc6, and Pla2g2a (32–39). Conceptually, the method of CTL immunoselection followed by LOH analysis described in this report is particularly amenable to identifying genes that, when mutated, contribute to but are not critical for the transformed state and, thus, can be lost by the tumor when subjected to negative selection. For example, Aldaz et al. (40) propose that a tumor suppressor gene localized to this region of distal chromosome 4 is more frequently lost in advanced tumors than in primary tumors. MCA-induced mutations in such a suppressor gene could act in a dominant or semidominant manner to promote tumorigenesis. As noted by Klein (41), a key question is whether the diversity of TSAs is a reflection of an even greater diversity of mutated gene products, only a small subset of which contribute to carcinogenesis, or whether TSAs mark a special subset of genes with mutations that allow tumor growth and progression. The localization of the Ram1 tumor Ag gene raises the possibility that TSAs may reveal a special subset of genes whose altered products play an accessory role in tumor biology.

It is notable that 16 escape variants did not manifest LOH when examined using any of the markers surveyed. Thus, these variants lost the Ram1 epitope by mechanisms undetectable by LOH analysis at the resolution used in this study. We have previously observed that such Ag-loss variants commonly carry micro-LOH or changes that lead to allele-specific expression defects in the Ag-encoding gene (7). Characterization of the genetic changes leading to the Ag-loss phenotype in these variants should aid in identifying the Ram1 gene.

References

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