[CANCER RESEARCH 60, 3584–3591, July 1, 2000]

# Serological Cloning of a Melanocyte *rab* Guanosine 5′-Triphosphate-binding Protein and a Chromosome Condensation Protein from a Melanoma Complementary DNA Library[1]

**Dirk Jäger, Elisabeth Stockert, Elke Jäger, Ali O. Güre, Matthew J. Scanlan, Alexander Knuth, Lloyd J. Old, and Yao-Tseng Chen[2]**

*Department of Pathology, Weill Medical College of Cornell University (D. J., Y.-T. C.), and Ludwig Institute for Cancer Research, New York Branch at Memorial Sloan-Kettering Cancer Center (E. S., A. O. G., M. J. S., L. J. O.), New York, New York 10021; and Medizinische Klinik, Hämatologie-Onkologie, Krankenhaus Nordwest, 60488 Frankfurt, Germany (D. J., E. J., A. K.)*

## ABSTRACT

**Characterization of immunogenic human melanoma antigens has been a major focus of tumor immunologists over the past two decades, and a broad array of antigens recognized by antibodies and T cells in the autologous host has been defined. In the present study, a melanoma library was screened by SEREX (serological analysis of cDNA expression libraries), and 43 genes were isolated, 2 of which, *NY-MEL-1* and *NY-MEL-3*, encode novel gene products with differential tissue expression. *NY-MEL-1* encodes a new rab GTP-binding protein, rab38. Among >40 rab proteins, rab38 has a unique COOH terminus which would allow posttranslational farnesylation and palmitoylation, lipid modifications normally occurring in ras proteins but not in other rab proteins. It is also the only *rab* gene showing a predominant mRNA expression in melanocytes, a cell-specific expression pattern likely related to melanosomal transport and docking. Northern blot analysis showed no detectable expression in other normal tissues. Consistent with this lineage specificity, rab38 mRNA is expressed in 80–90% of melanoma (17 of 19), but rarely in nonmelanocytic malignancies (1 of 16). The second novel gene isolated, *NY-MEL-3*, encodes a mitotic protein highly homologous to the *Xenopus* chromosome condensation protein XCAP-G, designated hCAP-G. Analysis of hCAP-G mRNA expression showed highest expression in the testis among normal tissues and variable expression in tumor cells, reflecting the proliferative activity in these cells. This mitosis-related expression suggests hCAP-G as a possible proliferation marker and a potential prognostic indicator in cancer. These findings provide further support that SEREX can define biologically significant molecules in cancer.**

## INTRODUCTION

The antigenic profile of human melanoma has been the object of intense scrutiny, motivated mainly by the desire to find antigens that can serve as suitable targets for vaccine or antibody-based therapies (1–4). Several general approaches to defining melanoma antigens have been established, and this has led to an impressive list of human melanoma antigens recognized by antibodies or T cells in humans or monoclonal antibodies generated in mice (3, 4). With regard to melanoma antigens recognized by humans, the approach of autologous typing provided the framework for analyzing the specificity of humoral (5) or cytotoxic T cell (6) reactivity against autologous melanoma cells. This led to the molecular characterization of CTL[3]-recognized peptides by the methodology developed by Boon and van der Bruggen (1) and antibody-recognized antigens according to SEREX methodology by Sahin *et al.* (7). The analysis of surface and intracellular antigens by mouse monoclonal antibodies represents another approach to defining the antigenic phenotype of melanoma and a wide range of melanoma differentiation antigens, including gp75 (8) and gp100 (9), have been identified this way.

The current list of restricted melanoma antigens recognized by these methods falls into three main categories. The first are melanocyte differentiation antigens, including tyrosinase (10), gp75 (11), gp100 (12), and Melan-A/MART-1 (13, 14). Of these, tyrosinase, gp75, and gp100 are melanosome-associated transmembrane proteins involved in the melanin synthesis pathway. These three proteins are structurally related, and gp75 and gp100 have been designated as tyrosinase-related proteins 1 and 2, respectively. In contrast, Melan-A is structurally unrelated, and its function is unknown. The second category comprises mutational antigens and a growing list of mutations in melanoma cells giving rise to CTL-recognized epitopes are being defined (15). The third category of melanoma antigens are the CT antigens (16), including MAGE, BAGE, GAGE, SSX, NY-ESO-1, CT7, and CT10 (1, 16). Like melanocyte differentiation antigens, these antigens are highly restricted differentiation antigens, but in the case of CT antigens they are normally expressed only in the germ cells of the testis. In cancer, CT antigens are aberrantly expressed in a wide range of different tumor types, including melanoma. The expression patterns of melanocyte differentiation antigens and CT antigens in melanoma are quite distinct. Melanocyte differentiation antigens are expressed at high frequency, with >80% of all melanomas expressing these antigens, although with substantial intratumor heterogeneity (9, 17). As expected, melanocyte differentiation antigens are generally found in melanomas with more differentiated phenotype and absent in less differentiated variants, such as the desmoplastic and spindle cell variants (18). In contrast, CT antigens are expressed at lower frequencies, in the range of 10–30% for individual CT antigens (15). In the case of MAGE antigens, there is more frequent expression in metastatic melanoma than in primary melanoma (19), suggesting a possible relation to tumor progression.

Regarding immune recognition of these antigens in humans, tyrosinase, gp75, gp100, and Melan-A/MART-1 have been shown to be recognized by $CD8^+$ T cells (15). Tyrosinase also elicited a $CD4^+$ T cell response (20) and an antibody response (7). In the CT antigen category, NY-ESO-1 is often recognized by both humoral and cellular immunity (21), whereas MAGE, BAGE, and GAGE, initially defined as $CD8^+$ CTL antigens, rarely elicit spontaneous antibody responses (22). SSX, CT7, and CT10, identified more recently by SEREX, have not as yet been shown to be CTL targets. Recently, a series of MAGE-3 peptides recognized by $CD4^+$ T cells has been defined (23).

SEREX analysis of melanoma by Sahin *et al.* (7) defined 10 antigens, including MAGE-1, SSX2, and tyrosinase. In subsequent SEREX studies, sera from melanoma patients were screened against a testicular cDNA library (24) and an allogeneic melanoma cell line library (25). The testicular library screening led to the definition of the SSX gene family, and the cell line library screening led to the

Received 12/16/99; accepted 4/27/00.

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 partially supported by NIH Grant CA-68024 and by the Cancer Research Institute/Rhea (Rose Marie) Finnell Memorial Fellowship.

[2] To whom requests for reprints should be addressed, at Department of Pathology, Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10021. Phone: (212)746-6472; Fax: (212)746-4483; E-mail: ytchen@mail.med.cornell.edu.

[3] The abbreviations used are: CTL, cytotoxic T cell; SEREX, serological analysis of recombinant tumor cDNA expression libraries; CT antigen, cancer-testis antigen; RT, reverse transcription; EST, expressed sequence tag.

Table 1 *Known genes identified by autologous SEREX screening of MZ19 cDNA library*

| Designation | No. of clones | UniGene No. | GenBank No. | Gene |
|---|---|---|---|---|
| *NY-MEL-1* | 1 | NA[a] | M94043 | rab-related GTP-binding protein homologue |
| *NY-MEL-2* | 1 | Hs. 111334 | AF109718 | Subtelomeric region chromosome 3 |
| *NY-MEL-3* | 1 | Hs. 193602 | AF111423 | XCAP-G homologue |
| *NY-MEL-4* | 1 | Hs. 78202 | D26156 | Transcriptional activator hSNF2b |
| *NY-MEL-5* | 3 | Hs. 73798 | NM002415 | Macrophage migration-inhibiting factor |
| *NY-MEL-6* | 1 | Hs. 129887 | AF047826 | Cadherin 7 |
| *NY-MEL-7* | 1 | Hs. 181349 | AF091083 | Clone 628, chromosome X |
| *NY-MEL-8* | 2 | Hs. 245188 | NM000362 | Tissue inhibitor of metalloproteinase 3 |
| *NY-MEL-9* | 1 | Hs. 217493 | NM004039 | Lipocortin II |
| *NY-MEL-10* | 1 | Hs. 4791 | AB002374 | KIAA 0376 |
| *NY-MEL-11* | 1 | Hs. 180909 | NM002574 | Proliferation-associated gene |
| *NY-MEL-12* | 2 | Hs. 194625 | NM006141 | Dynein light intermediate chain 2 |
| *NY-MEL-13* | 1 | Hs. 2953 | NM001019 | Ribosomal protein S15a |
| *NY-MEL-14* | 1 | Hs. 206908 | Z21852 | HERV-K LTR |
| *NY-MEL-15* | 1 | Hs. 180532 | X15183 | HSP 90 |
| *NY-MEL-16* | 1 | Hs. 78829 | D80012 | KIAA 0190 |
| *NY-MEL-17* | 1 | Hs. 180532 | X07872 | HSP 86 |
| *NY-MEL-18* | 1 | Hs. 168655 | L07872 | JK-recombination signal-binding protein |
| *NY-MEL-19* | 1 | Hs. 154854 | NM006227 | Phospholipid transfer protein |
| *NY-MEL-20* | 4 | Hs. 155344 | NM004401 | DNA fragmentation factor, 45 kDa, $\alpha$-subunit |
| *NY-MEL-21* | 1 | Hs. 184109 | L22154 | Ribosomal protein L37a |
| *NY-MEL-22* | 1 | Hs. 28190 | NM006395 | Ubiquitin-activating enzyme E1-like protein |
| *NY-MEL-23* | 1 | Hs. 89643 | NM001064 | Transketolase |
| *NY-MEL-24* | 9 | Hs. 54971 | U11687 | 30S ribosomal protein S1 homologue |
| *NY-MEL-25* | 1 | Hs. 169793 | NM000994 | Ribosomal protein L32 |
| *NY-MEL-26* | 1 | Hs. 5912 | Z71183 | Cosmid N28H9 chromosome 22q11.2 |
| *NY-MEL-27* | 1 | Hs. 10842 | AF054183 | GTP-binding protein mRNA |
| *NY-MEL-28* | 1 | Hs. 118787 | NM000358 | TGF-$\beta$-induced gene product |
| *NY-MEL-29* | 1 | Hs. 4311 | AF090384 | SUMO-1 |

[a] NA, not applicable; TGF-$\beta$, transforming growth factor $\beta$.

identification of CT7 (25). In the present study, we further extended our study and screened an autologous melanoma cell line library. Forty-three gene products were identified, including a rab GTP-binding protein preferentially expressed in melanocytes and a novel human chromosome condensation protein hCAP-G.

## MATERIALS AND METHODS

**Cell Lines and Serum.** The melanoma cell line MZ19 as well as the autologous melanoma serum were derived from a 50-year old female patient at Krankenhaus Nordwest, Frankfurt, Germany. The cell line, derived from a metastatic lesion, was recognized by $CD8^+$ T cells from the same patient. Other melanoma cell lines (SK-MEL series) and breast cancer cell lines were obtained from the repository maintained at the Ludwig Institute for Cancer Research, New York Branch at the Memorial Sloan-Kettering Cancer Center.

**RNA Extraction and Construction of cDNA Expression Library.** Total RNA was extracted from the MZ19 melanoma cell line by conventional CsCl-guanidine thiocyanate gradient method. A cDNA library was constructed in a λ-ZAP express vector, using a commercial cDNA library kit (Stratagene).

**Immunoscreening of the cDNA Library.** The unamplified cDNA expression library was screened with the autologous serum at 1:200 dilution. The screening procedure was as described previously (7, 26).

**Sequence Analysis of the Reactive Clones.** The reactive clones were purified and *in vivo* excised to pBK-CMV plasmid forms (Stratagene). Plasmid DNA was prepared and sequenced (DNA Sequencing Service, Cornell University, Ithaca, NY). DNA and amino acid sequences were analyzed against GenBank and EST databases using the BLAST program. Genes identical with entries in the GenBank were classified as known genes, whereas those that shared sequence identity only with ESTs and those that have no identity in both GenBank and EST databases were designated as unknown genes.

**RT-PCR.** To evaluate the mRNA expression pattern of the cloned cDNA in normal and malignant tissues, gene-specific oligonucleotide primers were designed to amplify cDNA segments 300–600 bp long. RT-PCR was performed by using 30 amplifications at an annealing temperature of 60°C, and the products were analyzed by agarose gel electrophoresis.

**Northern Blot Analysis.** Northern blot analysis was performed using commercial poly(A) (2 $\mu$g/lane) Human Multiple Tissue Northern (MTN) Blot I and II (Clontech), or total RNA (20 $\mu$g/lane) derived from different melanoma cell lines. $^{32}$P-labeled PCR probes 300–600 bp long were used, and the blots were hybridized and washed following the manufacturer's protocol (ExpressHyb kit; Clontech).

## RESULTS

A cDNA library of $7 \times 10^5$ primary clones was prepared from melanoma cell line MZ19 and screened with autologous serum at 1:200 dilution. Sixty-four immunoreactive clones were purified and DNA sequenced. Sequence comparison against GenBank and EST databases showed that these 64 clones were derived from 43 distinct genes, designated *NY-MEL-1* through *NY-MEL-43*. Among these, 29 genes showed sequences identical with or highly homologous to known GenBank entries. These genes are referred to as "known genes" and are summarized in Table 1. The remaining 14 genes showed no homologous sequences in the GenBank but shared sequences with ESTs derived from various tissues. These 14 genes, referred to as "unknown genes," are summarized in Table 2.

**mRNA Expression of SEREX-defined Genes from MZ19 Cells.** Of 29 previously known genes, 22 genes (*NY-MEL-8* to *NY-MEL-29)* are broadly expressed in human tissues, based on the finding that cDNAs derived from various tissues could be found in the GenBank and EST databanks. Similarly, 10 of 14 unknown genes have ESTs isolated from a number of normal tissues, presumably also reflecting ubiquitous mRNA expression in adult tissues. Such universal expression, however, was not immediately evident for seven known genes and four unknown genes, and the expression of these genes were evaluated by RT-PCR analysis of total RNA from five representative normal tissues (brain, colon, kidney, testis, and liver), using the isolated clone as the positive control. Results showed universal expression at similar levels with four of four unknown genes (*NY-MEL-30* to *NY-MEL-33)* and five of seven known genes (*NY-MEL-2*, and *NY-MEL-4* to *NY-MEL-7*; see Table 1). With two genes, *NY-MEL-1* and *NY-MEL-3*, a pattern of differential tissue expression was seen. *NY-MEL-1* (homologous to rat rab-related GTP-binding protein) showed a strong RT-PCR signal in MZ19 cells, weaker signals in testis and kidney, and was negative in brain, colon, and liver. *NY-*

Table 2 *Unknown genes identified by autologous SEREX screening of MZ19 cDNA library*

| Designation | No. of clones | EST sources/RT-PCR results |
|---|---|---|
| *NY-MEL-30* | 4 | EST: total fetus, melanocytes; RT-PCR: ubiquitous[a] |
| *NY-MEL-31* | 1 | EST: brain, mammary gland, parathyroid tumor, colon; RT-PCR: ubiquitous |
| *NY-MEL-32* | 1 | EST: brain, pregnant uterus; RT-PCR: ubiquitous |
| *NY-MEL-33* | 1 | EST: tonsils (germinal center B cell enriched), melanocytes; RT-PCR: ubiquitous |
| *NY-MEL-34* | 1 | EST: pregnant uterus, infant brain, fetal heart, CLL[b], eye, aorta, breast |
| *NY-MEL-35* | 1 | EST: melanocytes, Wilms' tumor, tonsil, adrenal gland, fetal spleen |
| *NY-MEL-36* | 1 | EST: prostate, fetal liver, colon tumor |
| *NY-MEL-37* | 1 | EST: cerebellum, mouse skin, mouse mammary gland |
| *NY-MEL-38* | 1 | EST: HeLa cells, mouse liver |
| *NY-MEL-39* | 1 | EST: colon, ovarian cancer, testis, fibroblast |
| *NY-MEL-40* | 1 | EST: brain, retina, placenta, prostate cancer, pregnant uterus |
| *NY-MEL-41* | 1 | EST: spleen, neuron, liver, Wilms' tumor |
| *NY-MEL-42* | 1 | EST: kidney, melanocytes, breast, testis |
| *NY-MEL-43* | 1 | EST: pregnant uterus, glioblastoma, pancreatic islets |

[a] Universal expression at similar levels in a normal tissue panel (brain, kidney, liver, colon, and testis); see text.
[b] CLL, chronic lymphocytic leukemia.

*MEL-3* (homologous to *Xenopus* chromosome-associated peptide G) showed strong RT-PCR signals in MZ19 and testis, with positive but weaker signals seen in brain, colon, liver, and kidney (data not shown).

**Predominant NY-MEL-1 Expression in Melanocytic Lineage.** mRNA expression of *NY-MEL-1* was evaluated in a larger panel of normal tissues by Northern blot analysis. Northern blotting with 2 $\mu$g of poly(A) RNA and a 5′ NY-MEL-1 probe showed no visible signal in any of the normal tissues tested, including heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocyte (Fig. 1*a*). Control hybridization with actin probe confirmed the quality and quantity of the poly(A) RNA (Fig. 1*b*). Expression in cells of melanocytic lineage was then examined, using total RNA from cultured melanocytes and eight melanoma cell lines (20 $\mu$g each, equivalent to $<1$ $\mu$g of poly(A) RNA). Results showed a single strong mRNA species migrating at ~1.6 kb in cultured melanocytes and variable but weaker signals in melanoma cell lines SK-MEL-12, -14, -26, -28, and -37. No detectable NY-MEL-1 mRNA was found in SK-MEL-10, -24, or -MZ19 after overnight exposure (Fig. 1*c*). A weak signal was visualized in MZ19 after 1 week of exposure, indicating a lower level expression then in SK-MEL-10 and SK-MEL-24. This predominant expression in melanocyte and melanoma cells but not in other normal tissues indicates that NY-MEL-1 belongs to the category of melanocyte differentiation antigens.

To analyze NY-MEL-1 mRNA expression in a larger panel of melanoma and other tumor samples, RT-PCR assay was performed and compared with the Northern blot data. Of the eight melanoma cell lines tested, seven were positive by RT-PCR, including SK-MEL-12, -14, -26, -28, and -37 (Northern blot positive), SK-MEL-10 (Northern

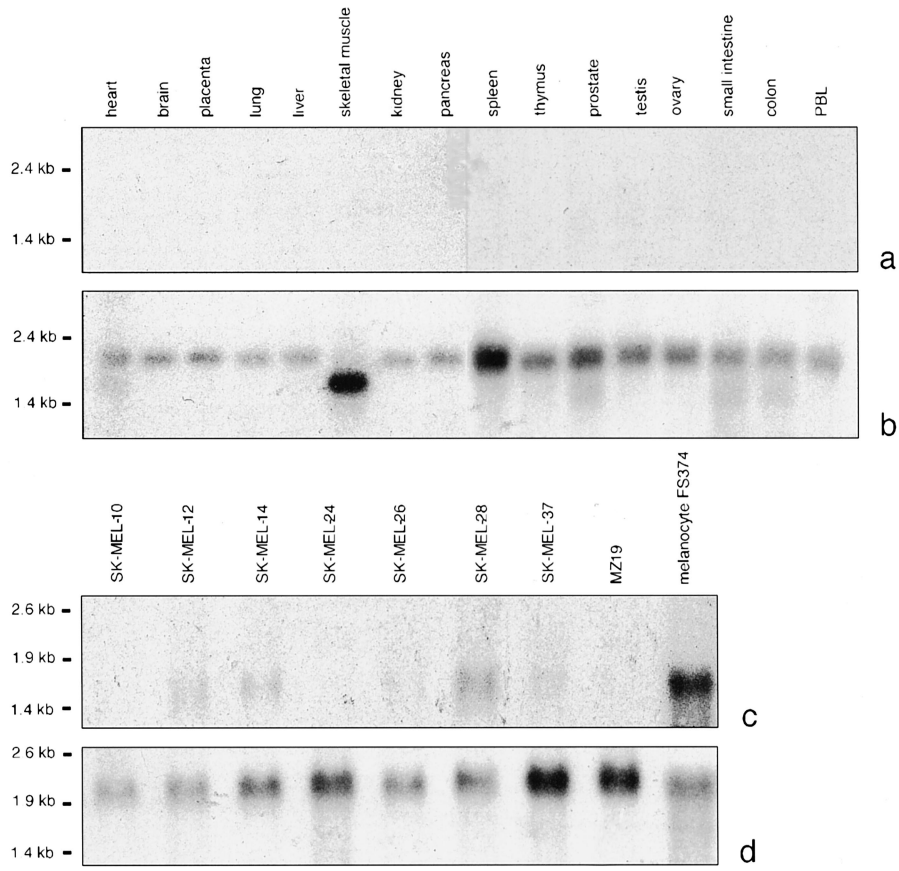


Fig. 1. Northern blot analysis of *NY-MEL-1* expression in normal tissues, cultured melanocytes, and melanoma cell lines. Poly(A) RNA, 2 $\mu$g/lane, was used for normal tissue blot (*a* and *b*), and 20 $\mu$g of total RNA was used for the melanocyte and melanoma cell line blot (*c* and *d*). Blots were initially hybridized with a PCR-derived *NY-MEL-1* probe, showing no detectable signal in the normal tissues (*a*), a strong mRNA species at ~1.6 kb in melanocyte, and similar but weaker signals in five of seven melanoma lines (SK-MEL-12, -14, -26, -28, and -37; *c*). The same blots were stripped of the probe and rehybridized with actin probe to confirm the RNA quantity and quality (*b* and *d*).

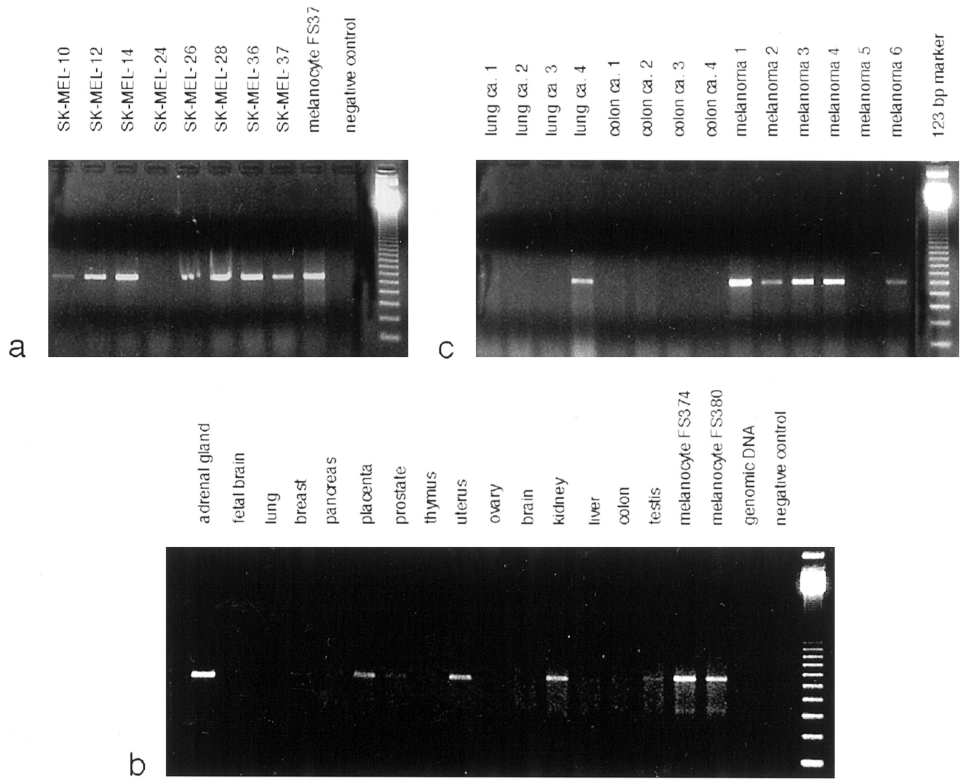


Fig. 2. RT-PCR analysis of *NY-MEL-1* expression in normal and tumor tissues and cells. *A, NY-MEL-1* expression in melanocyte and in seven of eight melanoma cell lines, except for SK-MEL-24. Normal tissue panel *(b)* shows expression in adrenal gland and several other tissues, most of them at levels lower than those of the two control melanocyte preparations (FS374 and FS380). The possibility of genomic DNA amplification was excluded by negative genomic DNA lane. *C*, examples of *NY-MEL-1* expression in tumors, revealing *NY-MEL-1* expression in five of six melanomas, and in a case of lung cancer. Seven other nonmelanocytic cancers were negative.

blot negative), and SK-MEL-36 (not tested by Northern blot; Fig. 2*a*). Two cultured melanocyte preparations were both strongly positive by RT-PCR. SK-MEL-24, the only melanoma line negative by RT-PCR, was also negative by Northern blot. Six breast cancer cell lines, 734B, MDA-MB-444, HBL 100, BT20, ZR71-1, and MDA-MB-231, were also RT-PCR negative. In accordance with the greater sensitivity of RT-PCR, several Northern blot-negative tissues showed weak to moderate RT-PCR signals, including testis, kidney, uterus, prostate, and pancreas. Adrenal gland, a tissue source not included in our Northern blot panel, showed a strong RT-PCR signal comparable with that of cultured melanocytes. This product was confirmed to be NY-MEL-1 by nested PCR with internal primers and by sequencing of the RT-PCR product.

A panel of tumor tissues was then examined by RT-PCR. Of 19 melanoma tumor specimens, 17 (90%) were positive, with moderate to strong signals seen in 12 (63%) cases. Nonmelanocytic tumors tested included 4 colon cancer, 4 lung cancer, 4 breast cancer, and 4 squamous carcinomas. All 16 specimens were negative, except for 1 lung cancer (lung ca 4; Fig. 2).

**NY-MEL-1 Encodes a Rab-related GTP-binding Protein.** DNA sequencing of *NY-MEL-1*, represented by cDNA clone MZ19–32a, revealed a full-length cDNA clone of 1407 bp, including 47 bp of 5′-untranslated region, 724 bp of 3′-untranslated region, and an open reading frame of 636 bp, encoding a polypeptide of 211 amino acids, a predicted molecular mass of 23.714 kDa (Fig. 3, GenBank number AF235022). Protein motif analysis showed that the five highly conserved GTP-binding domains that are typical for the ras superfamily (ras, rab, rho, ran, arf) of small GTP-binding proteins (26) are present in this sequence, as highlighted in Fig. 3.

Sequence comparison with the GenBank database confirmed that this gene is highly homologous to the rat rab-related GTP-binding protein, sharing 81.5% nucleotide identity and 97% amino acid identity. Rab GTP-binding proteins have been shown to play a crucial role in the intracellular processes of membrane trafficking and vesicular fusion and targeting (28). More than 40 rab GTP-binding proteins have been isolated from mammalian cells (28), at least 28 from human cells. Among human rabs, *NY-MEL-1* is closest to *rab32* at both DNA and protein levels (GenBank NM006834), sharing 75% amino acid sequence identity, 88% homology including conservative changes. On the basis of these findings, *NY-MEL-1* was designated as *rab38* (International System for Gene Nomenclature gene symbol: *RAB38*). Fig. 4 shows the amino acid sequence comparison of *rab38*, its rat homologue gene, and the human *rab32*.

Despite its overall strong homology to rab proteins, rab38 is unique in its COOH terminus. Rab proteins typically contain two cysteines in the carboxyl end, with a motif of -CC, -C*X*C, or -CC*XX*. This allows posttranslational lipid modification, most likely in the form of geranylgeranyl prenylation of both cysteine residues (29). Such hydrophobic modification enables the rab protein to interact with lipid membranes and is functionally crucial. Rab38, however, ends with -CSGCAKS, a sequence closer to ras than to rab (30). This primary structure would dictate a different pattern of lipid modification, likely farnesyl prenylation at the carboxycysteine residue and a palmitoylation at the upstream cysteine, as has been shown in ras protein (30).

**NY-MEL-3 Encodes a Mitosis-related Chromosome Condensation Protein.** In addition to *NY-MEL-1*, the only other SEREX-defined gene in this study that showed a differential expression pattern in normal tissues is *NY-MEL-3*, represented by clone MZ19–59b. This clone was completely sequenced, revealing a full-length cDNA of 3198 bp, including 68 bp of 5′-untranslated region, 82 bp of 3′-untranslated region, and an open reading frame of 3048 bp, encoding a polypeptide of 1015 amino acids, predicted molecular mass 114.3 kDa (submitted to GenBank, accession number AF235023).

A sequence homology search showed *NY-MEL-3* to be the human counterpart of *Xenopus laevis* chromosome-associated polypeptide group G (XCAP-G; GenBank accession number AF111423), a chromosome condensation protein which is part of the 13S condensin complex formed during the early stage of mitosis. *NY-MEL-3* shares

```
GGCTGCGCTTCCCTGGTCAGGCACGGCACGTCTGGCCGGCCGCCAGGATGCAGGCCCCGCACAAGGAGCACCTGTACAAGTTGCTGGTGATTGGCGACCT   100
                                                M  Q  A  P  H  K  E  H  L  Y  K  L  L  V  I  G  D  L    18

GGGCGTGGGGAAGACCAGTATCATCAAGCGCTACGTGCACCAGAACTTCTCCTCGCACTACCGGGCCACAATCGGCGTGGACTTCGCGCTCAAGGTGCTC   200
  G  V  G  K  T  S  I  I  K  R  Y  V  H  Q  N  F  S  S  H  Y  R  A  T  I  G  V  D  F  A  L  K  V  L    51

CACTGGGACCCGGAGACTGTGGTGCGCCTGCAGCTCTGGGATATCGCAGGTCAAGAAAGATTTGGAAACATGACGAGGGTCTATTACCGAGAAGCTATGG   300
 H  W  D  P  E  T  V  V  R  L  Q  L  W  D  I  A  G  Q  E  R  F  G  N  M  T  R  V  Y  Y  R  E  A  M     84

GTGCATTTATTGTCTTCGATGTCACCAGGCCAGCCACATTTGAAGCAGTGGCAAAGTGGAAAAATGATTTGGACTCCAAGTTAAGTCTCCCTAATGGCAA   400
G  A  F  I  V  F  D  V  T  R  P  A  T  F  E  A  V  A  K  W  K  N  D  L  D  S  K  L  S  L  P  N  G  K   118

ACCGGTTTCAGTGGTTTTGTTGGCCAACAAATGTGACCAGGGGAAGGATGTGCTCATGAACAATGGCCTCAAGATGGACCAGTTCTGCAAGGAGCACGGT   500
  P  V  S  V  V  L  L  A  N  K  C  D  Q  G  K  D  V  L  M  N  N  G  L  K  M  D  Q  F  C  K  E  H  G    551

TTCGTAGGATGGTTTGAAACATCAGCAAAGGAAAATATAAACATTGATGAAGCCTCCAGATGCCTGGTGAAACACATACTTGCAAATGAGTGTGACCTAA   600
 F  V  G  W  F  E  T  S  A  K  E  N  I  N  I  D  E  A  S  R  C  L  V  K  H  I  L  A  N  E  C  D  L    184

TGGAGTCTATTGAGCCGGACGTCGTGAAGCCCCATCTCACATCAACCAAGGTTGCCAGCTGCTCTGGCTGTGCCAAATCCTAGTAGGCACCTTTGCTGGT   700
M  E  S  I  E  P  D  V  V  K  P  H  L  T  S  T  K  V  A  S  C  S  G  C  A  K  S                       211

GTCTGGTAGGAATGACCTCATTGTTCCACAAATTGTGCCTCTATTTTTACCATTTTGGGTAAACGTCAGGATAGATATACCACATGTGGCAAGCCAAAGA   800

TCTATGCCTCTGTTTTTTCAATGAGAGAGAAATAGCAAATGTTCTTTCTATGCTTTCCTCACCATCATCACAGTGTTTACAAACTTTTGAAAATATTTAG   900

TCTGTTACAAACTTCTGTCATGTAGCTGACCAAAATCCTGCAGGGCCACAGTCGGCACTGTTATTTGCTTCTTTTAATCAGCAAAGGCCTCAAGTCTTAA  1000

AATAAAAGGGGAGAAGAACAAACTAGCTGTCAAGTCAAGGACTGGCTTTCACCTTGCCCTGGTGTCTTTTTCCAGATTTCAATATATTCTCTGATGGCCT  1100

GACAGGCCTATTAAGTAGATGTGATATTTTCTTCCAAGATGACCTCCATTCTCGGCAGACCTAAGAGTTGCCTCTGAGTTAGCTCTTTGGAATCGTGAAC  1200

ACAGGTGTGCTATATTGTCCTTGTCCTAACTGTCACTTGCCATGGCCTGAATGTTGGCTTAACTGAATATTGTATGAAAAGACATGCCTCCATATGTGCC  1300

TTTCTGTTAGCTCTCTTTGACTCAAGCTGTGGGGCTCCTCTATACATGCTATACATGTAATATATATTATATATATTTTTGCAAGTGAACAATAAAACAT  1400

TAAAAGATAAAA                                                                                          1412
```

Fig. 3. Nucleotide and amino acid sequences of NY-MEL-1. The five domains (G1 to G5) that form the guanine nucleotide-binding site are underlined. The highly conserved consensus residues within each domain are shown in *boldface* and are as follows: G1: G*XXXX*GK(S/T), G2: *XXXXTXXX*, G3: *XX*D*XX*G*XX*, G4: *XX*NK*X*D, and G5: *XXX*(T/G)(C/S)A*X*.

28% nucleotide identity and 59% amino acid identity with XCAP-G, and we have therefore designated this gene *hCAP-G*.

Expression of *hCAP-G* in normal and tumor cells was evaluated by RT-PCR and by Northern blot analysis. Northern blotting of normal tissue RNAs showed the strongest expression in testis, a weak signal in thymus, and no detectable signal in other normal tissues (Fig. 5). Melanoma cell line RNAs (20 $\mu$g of total RNA) showed variable signals of weak to moderate intensity. RT-PCR was performed for normal tissues, melanoma cell lines, and melanoma tumor samples. All RNA tested were positive, with highly variable levels of RT-PCR

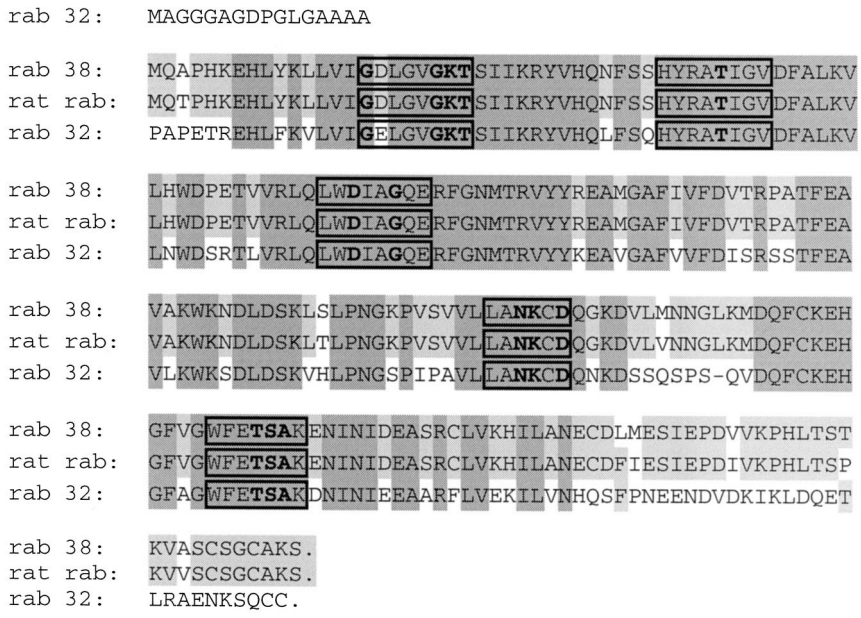


Fig. 4. Comparison of NY-MEL-1/rab38 amino acid sequence with the rat homologue gene and the closest human *rab*, *rab32*. The five GTP-binding domains (*boxed*) showed near 100% homology, including all of the consensus residues (*boldface*) in the binding motifs. Sequences shared by all three *rab*s are shaded, and the ones shared by two *rab*s are in a lighter shade. *rab38* is highly homologous to its rat counterpart throughout the entire molecule, whereas the similarities between *rab38* and *rab32* disappear in the last 30 amino acids at the COOH termini (see text).

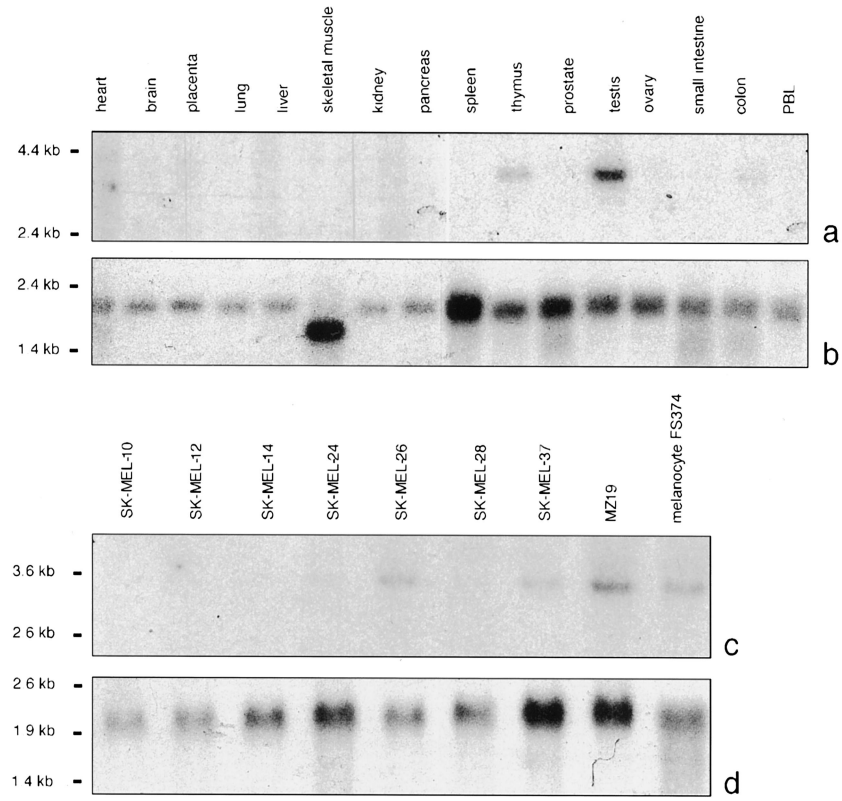


Fig. 5. Northern blot analysis of hCAP-G expression in normal tissues and melanoma cell lines. The same blots used for *NY-MEL-1* (Fig. 1) were reprobed with a PCR-derived *NY-MEL-3* probe (*a,* normal tissues*; c,* melanoma cell lines and melanocyte). A strong signal of ~3.5 kb is seen in testis, and a weaker signal is seen in thyroid. Other normal tissues show no detectable expression. Variable expression is seen in melanoma cell lines. RNA quantity and quality were confirmed with an actin probe (*b* and *d).*

products in these tissues and cell lines. The highest level of expression was seen in testis, as also observed in Northern blots.

**Presence of NY-MEL Sequences in SEREX Databank.** To compare the genes isolated in this NY-MEL series with those isolated from other SEREX studies, the sequences of *NY-MEL-1* through *NY-MEL-43* were analyzed against entries in the SEREX databank (http://www.licr.org/SEREX.html). Results showed that 8 of the 43 genes have previously been identified in the SEREX analysis of a range of tumor types, including renal cancer, colon cancer, gastric cancer, etc. (Table 3). *NY-MEL-14* shares 92% homology to a HERV-K long terminal repeat (GenBank Z21852) derived from an endogenous retroviral present in multiple copies in the human genome (31). HERV-K has been identified in a previous SEREX screening of renal cancer by Sahin *et al.* (32).

## DISCUSSION

Rab proteins, the largest branch of the ras superfamily of GTP-binding proteins, were first identified in yeast in 1983 (33). Search for the homologous genes in mammalian cells initially identified its counterpart in rat brain, hence the name rab (*ra*s-like in rat *b*rain). More than 40 rab genes have since been identified in mammals, including at least 28 human genes found in the public databanks. These genes share structural features with ras and other GTPases in having the 5 highly conserved regions necessary for GTP binding and hydrolysis. In addition, all rab proteins typically contain 2 cysteine residues in the carboxyl end, usually in the format of -C*X*C, -CC, or -CC*XY*. Isoprenylation of both cysteine residues by geranylgeranyltransferase renders the rab proteins hydrophobic, providing them the ability of reversible membrane association (34). Given this capacity, Rab protein functions by cycling between a GDP-bound cytosolic form and a GTP-bound membrane form, and various rab proteins have been shown to play a crucial role in the docking/fusion of transport vesicles or organelles with their target/acceptor membranes. Subcellular localization studies have indeed shown that with the exception of lysosomes, all of the organelles involved in biosynthetic/secretory and endocytic pathways contain at least one rab protein on their cytoplasmic surface (28).

Although most of the rab proteins identified are ubiquitously expressed in many tissues, some of them have been found to be cell type- or tissue-specific. For example, rab17 is detected only in epithelial cells (35), rab3a is expressed in neurons and neuroendocrine cells (36), and rab3d is mainly in adipocytes (36). It is likely that the cell-specific expression of these rab proteins is critical for cells to perform unique functions related to vesicle targeting and docking. In this regard, melanocytes might be suspected to have one or more unique rabs, because they contain a highly cell-specific organelle, the melanosome. Melanosomes are organelles that contain tyrosinase and other melanin synthesis-related proteins. As melanocytes mature, the

Table 3 *Genes identified in previous SEREX analysis*

| | |
|---|---|
| Lipocortin II | Thyroid cancer |
| KIAA 0376 | Renal cancer, colon cancer, breast cancer, pancreas cancer, hepatocellular carcinoma, lung cancer |
| HERV-K LTR | Renal cancer |
| HSP 90 | Renal cancer, breast cancer |
| HSP 86 | Renal cancer |
| JK-recombination signal-binding protein | Renal cancer, gastric cancer, breast cancer |
| Human phospholipid transfer protein | Hepatocellular carcinoma, colon cancer, gastric cancer, Hodgkin's disease, pancreatic cancer |
| Human DNA fragmentation factor | Hepatocellular carcinoma, colon cancer, prostate cancer, lung cancer |

melanosomes gradually move from the cytoplasm of the melanocytes toward the dendritic processes in the periphery. Morphological and biochemical changes occur during this process, defining melanosomal developmental stages I to IV. Mature melanosomes can then be transferred from the melanocytes to the adjacent keratinocytes as a result of active phagocytosis of the melanocytic dendrites by the keratinocytes (37). Considering this highly polarized movement of melanosomes and the eventual fusion and transfer to keratinocytes, it seems likely that unique rab proteins are involved in this process. This idea led to the isolation of an array of 17 rabs from melanocytes by a PCR-based strategy (38). Sequence analysis revealed a few new rab genes, including rab22b and rab30. However, none of the genes isolated in that study showed a melanocyte-specific expression pattern.

By screening the MZ19 melanoma cell line cDNA expression library with autologous antibody, we have identified *NY-MEL-1* in the present study, encoding a new member of the rab family protein. The closest human *rab* gene to *NY-MEL-1* is *rab32* which shows strong nucleotide and amino acid homology within and beyond the GTP-binding domains. For this reason, we designated the *NY-MEL-1* gene *rab38* following the recommendation of the International System for Gene Nomenclature.

At the RNA level, *rab38* is preferentially expressed in melanocytes and in their malignant counterpart, melanomas. When compared with other human *rab* gene sequences, *rab38* has a unique COOH terminus, -CSGCAKS, different from the -CC, -C*X*C, or -CC*XX* (*X* being any amino acid) motifs seen in other rab proteins (30). This sequence, in contrast, is similar to the -CAA*X* (A being an aliphatic residue, and *X* being M, S, Q, C, or A) motif seen in the ras subfamily proteins, which also contain an upstream cysteine residue as does rab38. It is known that posttranslational lipid modifications occur at both cysteine residues at the carboxy ends of the ras and rab proteins, allowing their hydrophobic interactions with the lipid membranes. However, the two cysteines in the rab proteins are geranylgeranylated, whereas the two cysteines in the ras protein are farnesylated (carboxycysteine) and palmitoylated (upstream cysteine), respectively. Whether these differences in lipid modification lead to specific interaction of rab38 with proteins related to melanosome trafficking is an idea worth exploring. Subcellular localization studies using anti-rab38 antibodies would be important to determine whether rab38 is indeed melanosome-associated. Two of our findings, however, suggest that rab38 may have a broader function besides its role in melanocytes. One is that the rat homologue gene has been isolated from lung alveolar cells, and the other is that *rab38* mRNA was detected at low levels in other normal tissues, and at substantial levels in the adrenal gland. Whether this indicates a leaky expression of *rab38* in many cell types or specific expression by a small subset of cells in these organs remains to be investigated.

Another novel human gene that we isolated by SEREX in the present study is the human counterpart of the *Xenopus* chromosome-associated polypeptide G (XCAP-G) gene, designated *hCAP-G*. This gene belongs to a family of SMC (structural maintenance of chromosomes) genes, which have been shown to be required for proper condensation and segregation of mitotic chromosomes (39, 40). By analyzing protein extracts of *Xenopus* oocytes, it has been shown that the chromosome condensation complex that forms during mitosis consists of two main fractions, the 13S condensin and the 8S condensin. The 13S condensin contains five main proteins, the XCAP-C, XCAP-E, XCAP-D2, XCAP-G, and XCAP-H. Together, these five proteins constitute the most abundant protein components besides histones and are considered central players in the mitotic protein assembly (41). By RT-PCR and Northern blot analysis of different normal and tumor tissues, we found that hCAP-G mRNA is highly expressed in normal testis, weaker (but detectable by Northern blot) in normal thymus, and at variable levels in different tumor specimens and tumor cell lines. Because hCAP-G presumably functions in mitosis and is cell cycle related, it might be expected that its expression shows a relationship with cell replication rate: highest in testis because of spermatogenesis; high in thymus as a lymphoid organ with constant cell turnover; and variable in tumor cells. Other genes involved in mitosis have been shown to be proliferation markers with prognostic significance in the evaluation of human malignancy. For example, high level expression of mitosin, a nuclear phosphoprotein involved in cell division (42), has been shown to be an independently significant predictor of recurrence in breast cancer without lymph node metastasis ("node-negative" breast cancer) (43). Another proliferative marker, Ki-67, has also been documented as clinically useful (44). In this regard, it would be important to compare the tumor expression of hCAP-G to the expression of these existing markers by immunohistochemical analysis, and antibodies to hCAP-G are being prepared.

The cloning of two biologically important genes, *rab38* and *hCAP-G*, by SEREX further attests to the power of this approach to identify novel human genes and gene products with potential relevance to cancer. SEREX-defined antigens can be categorized into several groups, including differentiation antigens, mutational antigens, amplified/overexpressed antigens, viral antigens, CT antigens, and splice-variant antigens (16). The immunogenicity of these antigens have been ascribed to tumor-specific expression (*e.g.*, mutational antigens), restricted expression (*e.g.*, differentiation antigens), or altered expression (*e.g.*, overexpressed antigens). In this context, rab38 is clearly a new differentiation antigen, mainly expressed in the melanocyte lineage, and hCAP-G would represent an overexpressed antigen in cancer.

To date, >1000 antigens have been isolated by SEREX analysis. The challenge of SEREX analysis is to define the complete repertoire of cancer gene products that elicit an immune response in humans (cancer immunome) and to distinguish those antigens that have a direct relevance to cancer etiology or cancer progression from those that represent general autoimmunogenic cellular components. Of the 43 genes isolated in the current study, 8 had been identified in previous SEREX analysis. This ~20% overlapping in sequences shows the progress that has been made in defining the cancer immunome, but also the work left to be done in completing the task.

## REFERENCES


1. Boon, T., and van der Bruggen, P. Human tumor antigens recognized by T lymphocytes. J. Exp. Med., *183:* 725–729, 1996.
2. Rosenberg, S. A. A new era for cancer immunotherapy based on the genes that encode cancer antigens. Immunity, *10:* 281–287, 1999.
3. Maeurer, M. J., Storkus, W. J., Kirkwood, J. M., and Lotze, M. T. New treatment options for patients with melanoma: review of melanoma- derived T-cell epitope-based peptide vaccines. Melanoma Res., *6:* 11–24, 1996.
4. Houghton, A. N., and Chapman, P. B. Clinical applications of monoclonal antibodies in cancer: melanoma. *In:* Biologic Therapy of Cancer, pp. 576–590. Philadelphia: J. B. Lippincott Co., 1995.
5. Old, L. J. Cancer immunology: the search for specificity—G. H. A. Clowes Memorial Lecture. Cancer Res., *41:* 361–375, 1981.
6. Knuth, A., Danowski, B., Oettgen, H. F., and Old, L. J. T-cell-mediated cytotoxicity against autologous malignant melanoma: analysis with interleukin 2-dependent T-cell cultures. Proc. Natl. Acad. Sci. USA, *81:* 3511–3515, 1984.
7. Sahin, U., Tureci, O., Schmitt, H., Cochlovius, B., Johannes, T., Schmits, R., Stenner, F., Luo, G., Schobert, I., and Pfreundschuh, M. Human neoplasms elicit multiple specific immune responses in the autologous host. Proc. Natl. Acad. Sci. USA, *92:* 11810–11813, 1995.
8. Mattes, M. J., Thomson, T. M., Old, L. J., and Lloyd, K. O. A pigmentation-associated, differentiation antigen of human melanoma defined by a precipitating antibody in human serum. Int. J. Cancer, *32:* 717–721, 1983.
9. Gown, A. M., Vogel, A. M., Hoak, D., Gough, F., and McNutt, M. A. Monoclonal antibodies specific for melanocytic tumors distinguish subpopulations of melanocytes. Am. J. Pathol., *123:* 195–203, 1986.

10. Brichard, V., Van Pel, A., Wolfel, T., Wolfel, C., De Plaen, E., Lethe, B., Coulie, P., and Boon, T. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J. Exp. Med., *178:* 489–495, 1993.
11. Wang, R. F., Robbins, P. F., Kawakami, Y., Kang, X. Q., and Rosenberg, S. A. Identification of a gene encoding a melanoma tumor antigen recognized by HLA-A31-restricted tumor-infiltrating lymphocytes. J. Exp. Med., *181:* 799–804, 1995.
12. Wang, R. F., Appella, E., Kawakami, Y., Kang, X., and Rosenberg, S. A. Identification of TRP-2 as a human tumor antigen recognized by cytotoxic T lymphocytes. J. Exp. Med., *184:* 2207–2216, 1996.
13. Coulie, P. G., Brichard, V., Van Pel, A., Wolfel, T., Schneider, J., Traversari, C., Mattei, S., De Plaen, E., Lurquin, C., Szikora, J. P., Renauld, J.-C., and Boon, T. A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J. Exp. Med., *180:* 35–42, 1994.
14. Kawakami, Y., Eliyahu, S., Delgado, C. H., Robbins, P. F., Rivoltini, L., Topalian, S. L., Miki, T., and Rosenberg, S. A. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc. Natl. Acad. Sci. USA, *91:* 3515–3519, 1994.
15. Van den Eynde, B. J., and van der Bruggen. P. T cell defined tumor antigens. Curr. Opin. Immunol., *9:* 684–693, 1997.
16. Old, L. J., and Chen, Y. T. New paths in human cancer serology. J. Exp. Med., *187:* 1163–1167, 1998.
17. Chen, Y. T., Stockert, E., Tsang, S., Coplan, K. A., and Old, L. J. Immunophenotyping of melanomas for tyrosinase: implications for vaccine development. Proc. Natl. Acad. Sci. USA, *92:* 8125–8129, 1995.
18. Jungbluth, A. A., Busam, K. J., Gerald, W. L., Stockert, E., Coplan, K. A., Iversen, K., MacGregor, D. P., Old, L. J., and Chen, Y. T. A103: an anti-melan-a monoclonal antibody for the detection of malignant melanoma in paraffin-embedded tissues. Am. J. Surg. Pathol., *22:* 595–602, 1998.
19. Brasseur, F., Rimoldi, D., Lienard, D., Lethe, B., Carrel, S., Arienti, F., Suter, L., Vanwijck, R., Bourlond, A., Humblet, Y., et al. Expression of MAGE genes in primary and metastatic cutaneous melanoma. Int. J. Cancer., *63:* 375–380, 1995.
20. Topalian, S. L., Rivoltini, L., Mancini, M., Markus, N. R., Robbins, P. F., Kawakami, Y., and Rosenberg, S. A. Human $CD4^+$ T cells specifically recognize a shared melanoma-associated antigen encoded by the tyrosinase gene. Proc. Natl. Acad. Sci. USA, *91:* 9461–9465, 1994.
21. Jager, E., Chen, Y. T., Drijfhout, J. W., Karbach, J., Ringhoffer, M., Jager, D., Arand, M., Wada, H., Noguchi, Y., Stockert, E., Old, L. J., and Knuth, A. Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. J. Exp. Med., *187:* 265–270, 1998.
22. Stockert, E., Jager, E., Chen, Y. T., Scanlan, M. J., Gout, I., Karbach, J., Arand, M., Knuth, A., and Old, L. J. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J. Exp. Med., *187:* 1349–1354, 1998.
23. Chaux, P., Vantomme, V., Stroobant, V., Thielemans, K., Corthals, J., Luiten, R., Eggermont, A. M., Boon, T., and van der Bruggen, P. Identification of MAGE-3 epitopes presented by HLA-DR molecules to CD4(+) T lymphocytes. J. Exp. Med., *189:* 767–778, 1999.
24. Gure, A. O., Tureci, O., Sahin, U., Tsang, S., Scanlan, M. J., Jager, E., Knuth, A., Pfreundschuh, M., Old, L. J., and Chen, Y. T. SSX: a multigene family with several members transcribed in normal testis and human cancer. Int. J. Cancer, *72:* 965–971, 1997.
25. Chen, Y. T., Gure, A. O., Tsang, S., Stockert, E., Jager, E., Knuth, A., and Old, L. J. Identification of multiple cancer/testis antigens by allogeneic antibody screening of a melanoma cell line library. Proc. Natl. Acad. Sci. USA, *95:* 6919–6923, 1998.
26. Chen, Y. T., Scanlan, M. J., Sahin, U., Tureci, O., Gure, A. O., Tsang, S., Williamson, B., Stockert, E., Pfreundschuh, M., and Old, L. J. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc. Natl. Acad. Sci. USA, *94:* 1914–1918, 1997.
27. Sprang, S. R. G protein mechanisms: insights from structural analysis. Annu. Rev. Biochem., *66:* 639–678, 1997.
28. Martinez, O., and Goud, B. Rab proteins. Biochim. Biophys. Acta, *1404:* 101–112, 1998.
29. Wilson, A. L., and Maltese, W. A. Coupled translation/prenylation of rab proteins *in vitro*. Methods Enzymol., *250:* 79–91, 1995.
30. Der, R. K., and Khosravi-Far, R. Prenylation analysis of bacterially expressed and insect cell-expressed Ras and Ras-related proteins. Methods Enzymol., *255:* 46–60, 1995.
31. Vinogradova, T., Volik, S., Lebedev, Y., Shevchenko, Y., Lavrentyeva, I., Khil, P., Grzeschik, K. H., Ashworth, L. K., and Sverdlov, E. Positioning of 72 potentially full size LTRs of human endogenous retroviruses HERV-K on the human chromosome 19 map: occurrences of the LTRs in human gene sites. Gene, *199:* 255–264, 1997.
32. Sahin, U., Tureci, O., and Pfreundschuh, M. Serological identification of human tumor antigens. Curr. Opin. Immunol., *9:* 709–716, 1997.
33. Gallwitz, D., Donath, C., and Sander, C. A yeast gene encoding a protein homologous to the human c-*has*/*bas* proto-oncogene product. Nature (Lond.), *306:* 704–707, 1983.
34. Rando, R. R. Chemical biology of isoprenylation/methylation. Biochem. Soc. Trans., *24:* 682–687, 1996.
35. Lutcke, A., Jansson, S., Parton, R. G., Chavrier, P., Valencia, A., Huber, L. A., Lehtonen, E., and Zerial, M. Rab17, a novel small GTPase, is specific for epithelial cells and is induced during cell polarization. J. Cell Biol., *121:* 553–564, 1993.
36. Baldini, G., Hohl, T., Lin, H. Y., and Lodish, H. F. Cloning of a Rab3 isotype predominantly expressed in adipocytes. Proc. Natl. Acad. Sci. USA, *89:* 5049–5052, 1992.
37. Schaumburg-Lever, W. F. L. A. G. Histology of the skin. *In:* Histopathology of the Skin, pp. 9–43. Philadelphia: J. B. Lippincott Co., 1990.
38. Chen, D., Guo, J., Miki, T., Tachibana, M., and Gahl, W. A. Molecular cloning of two novel *rab* genes from human melanocytes. Gene, *174:* 129–134, 1996.
39. Strunnikov, A. V., Larionov, V. L., and Koshland, D. SMC1: an essential yeast gene encoding a putative head-rod-tail protein is required for nuclear division and defines a new ubiquitous protein family. J. Cell Biol., *123:* 1635–1648, 1993.
40. Strunnikov, A. V., Hogan, E., and Koshland, D. SMC2, a *Saccharomyces cerevisiae* gene essential for chromosome segregation and condensation, defines a subgroup within the SMC family. Genes Dev., *9:* 587–599, 1995.
41. Hirano, T., Kobayashi, R., and Hirano, M. Condensins, chromosome condensation protein complexes containing XCAP- C, XCAP-E and a *Xenopus* homolog of the *Drosophila* Barren protein. Cell, *89:* 511–521, 1997.
42. Zhu, X., Mancini, M. A., Chang, K. H., Liu, C. Y., Chen, C. F., Shan, B., Jones, D., Yang-Feng, T. L., and Lee, W. H. Characterization of a novel 350-kilodalton nuclear phosphoprotein that is specifically involved in mitotic-phase progression. Mol. Cell. Biol., *15:* 5017–5029, 1995.
43. Clark, G. M., Allred, D. C., Hilsenbeck, S. G., Chamness, G. C., Osborne, C. K., Jones, D., and Lee, W. H. Mitosin (a new proliferation marker) correlates with clinical outcome in node-negative breast cancer. Cancer Res., *57:* 5505–5508, 1997.
44. Krecicki, T., Jelen, M., Zalesska-Krecicka, M., and Szkudlarek, T. Ki-67 immunostaining and prognosis in laryngeal cancer. Clin. Otolaryngol., *23:* 539–542, 1998.