A Marker for Neoplastic Progression of Human Melanocytes Is a Cell Surface Ectopeptidase

By Mark E. Morrison, Setaluri Vijayasaradhi, Dov Engelstein, Anthony P. Albino, and Alan N. Houghton

From the Memorial Sloan-Kettering Cancer Center, and Cornell University Medical College, New York, New York 10021

Summary

Adenosine deaminase binding protein (ADAbp) is a cell surface glycoprotein that is expressed by normal melanocytes but not by melanoma, the malignant counterpart. ADAbp is specifically downregulated during malignant transformation of melanocytes. Recently, we have developed a system that progressively transforms melanocytes in vitro in defined steps. Transduction with v-Ha-ras oncogene followed by long-term culture leads to a cell phenotype and genotype that specifically mimics human melanoma. Loss of ADAbp expression occurred concomitantly with the emergence of growth factor independence and appearance of specific chromosomal abnormalities. The cellular function of ADAbp has not been defined. To characterize ADAbp, the mature 110-kD form was purified from human kidney. Five tryptic peptides from purified human ADAbp revealed 100% homology to a serine protease, human dipeptidyl peptidase IV (DPP IV), also known as CD26. DPP IV activity was detected in lysates from human melanocytes and renal carcinoma cells but not melanoma cells, and DPP IV activity could be specifically isolated from melanocytes by binding to ADA or to S27 monoclonal antibody against ADAbp. These findings show that ADAbp is a cell surface ectopeptidase that is tightly regulated during neoplastic transformation of melanocytes.

Materials and Methods

Reagents. Con A-Sepharose, wheatgerm agglutinin (WGA)-Sepharose, and protein A-Sepharose were obtained from Pharmacia

1 Abbreviations used in this paper: ADAbp, adenosine deaminase binding protein; DPP IV, dipeptidyl peptidase IV; RAMPS, rabbit anti-mouse IgG protein A-Sepharose.

Melanoma is a neoplasm derived from cells of the melanocyte lineage. Malignant transformation of melanocytes leads to progressive and profound alterations in cell phenotype, manifested by invasion into surrounding tissues, dysregulated growth, and metastasis. Careful clinical and pathological analyses have led to the description of lesional steps in transformation and progression of human melanoma (1). Because of the accessibility of lesions that represent different stages in melanoma evolution, melanoma has become a useful paradigm to study progression of human cancer.

Some of the best characterized phenotypic changes observed during melanoma progression come from the study of antigen expression. A broad assortment of antigens, including intracellular and cell surface, have been defined on melanocytes and melanoma. Most antigens have been detected on both melanocytes and melanoma cells, and are presumably regulated during melanocyte differentiation. Several molecules or epitopes have been identified that discriminate steps in melanoma progression (2, 3). Despite the profound phenotypic alterations that occur during steps of tumor progression, however, it has been difficult to define qualitative changes (i.e., absence vs. presence) that absolutely distinguish normal cells in the melanocyte lineage from melanoma cells. Adenosine deaminase binding protein (ADAbp), a plasma membrane glycoprotein consisting of 110–120-kD subunits (4, 5), is a candidate for a molecule that is specifically regulated during melanocyte transformation and melanoma progression. We have previously found that ADAbp is consistently expressed by melanocytes in vitro and in vivo but cannot be detected on melanoma cells (6). Furthermore, transformation of melanocytes in vitro extinguishes ADAbp expression (6, 7). The present studies were undertaken to further explore the link between ADAbp expression and transformation of melanocytes. We show that ADAbp expression is lost as melanoma growth becomes independent of exogenous growth factors. ADAbp has been purified and shown to be indistinguishable from the cell surface serine protease, dipeptidyl peptidase IV (EC 3.4.14.5) (DPP IV).
carried out as previously described (6). Cells were labeled with washing, and the percentage of target cells exhibiting red cell ro-
incubated with serial dilutions of mouse mAb $27 for 1 h at room temperature. Target cells that were plated 2-3 d earlier were
viously described (6). Indicator cells were prepared by conjugation of rabbit anti-mouse IgG to human red blood cells with 0.01%
Glucosamine was obtained from New England Nuclear (Boston, MA). Standard reagents and buffers were obtained from Sigma Chemical Co. (St. Louis, MO). Microtest plates were obtained from Robins Scientific (Sunnyvale, CA).

Cell Lines and Tissues. Human melanoma and renal carcinoma cell lines were maintained in Eagle's MEM supplemented with 10% FCS, 0.1 mM nonessential amino acids, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Normal human melanocyte cell lines were maintained in Eagle's MEM supplemented with 10% FCS, 0.1 mM nonessential amino acids, 10 ng/ml PMA, 1 #g/ml cholera toxin, and 0.1% NP-40, 150 mM NaCl, and 1 mg/ml aprotinin. Bound proteins were eluted in 0.5-mI fractions of 100 mM glycine buffer, pH 2.5, containing 150 mM NaCl and 2 mg/ml aprotinin. Fractions were collected in 100 #l of 1 M Tris, pH 8.0 (final pH = 7.2). Aliquots of each fraction were analyzed by 7.5% SDS-PAGE under reducing conditions. Cells were silver stained to visualize protein. ADAbp-containing fractions were pooled. An aliquot was set aside for later purification by ADA-Affigel affinity matrix. The remainder of the ADAbp-containing fractions were precipitated by the addition of an equal volume of ice-cold acetone and stored overnight at -20°C. The precipitate was recovered by centrifugation and dissolved in sample buffer for SDS-PAGE. In parallel experiments, renal tissue was lysed and bound overnight to WGA-Sepharose. Bound glycoproteins were eluted with buffer containing 10% N-acetyl-glucosamine. Eluted material was incubated with mAb S27-Affigel and processed as above. An aliquot of ADAbp was further characterized by binding to ADA-Affigel. After binding overnight, the Affigel was washed with 30 vol of 50 mM Tris, pH 7.4, containing 0.1% NP-40, 0.5 M NaCl, and 1 mM EDTA, followed by low-salt buffer (150 mM NaCl). ADAbp was eluted with SDS-containing sample buffer and subjected to electrophoresis on a 7.5% polyacrylamide gel under reducing conditions. Proteins were visualized by silver stain.

Because renal tissue was not easily obtained, it was necessary to maximize the yield of ADAbp for use in amino acid analysis and sequence analysis of tryptic peptides. Material that did not bind to lectin under the stringent high-salt conditions described above was further purified by binding to mAb S27-Affigel affinity matrix and pooled with the ADAbp isolated above.

Amino Acid Analysis of ADAbp. An aliquot, representing 10% of the total mAb S27 affinity-purified ADAbp, was subjected to electrophoresis on a 0.75-mm thick 7.5% polyacrylamide gel under reducing conditions. ADAbp was electrotransferred onto Immobilon-P membrane in 10 mM 3-cyclohexylamino-1-propanesulfonic acid (CAPS) buffer, pH 11.0, containing 10% methanol (9). Transfer was complete after 75 min at 50 V (0.5 A). ADAbp was visualized by staining with 0.2% Ponceau S in 1% acetic acid. The ADAbp band was excised, destained in water, and allowed to air dry. Amino acid analysis was performed at the Harvard

Fine Chemicals (Piscataway, NJ). Affigel-10, Affigel-15, and electrophoresis molecular weight standards were obtained from Bio-Rad Laboratories (Richmond, CA). Rabbit anti-mouse IgG was obtained from Cappel Laboratories (Durham, NC). Rabbit anti-mouse IgG protein A-Sepharose (RAMPs) was prepared from rabbit anti-mouse IgG and protein A-Sepharose. S27 and S4 mouse mAbs were purified from ascites of hybridoma-bearing mice by binding to protein A-Sepharose. mAbs were >99% pure by SDS-PAGE. Bovine adenosine deaminase (ADA) was obtained from Boehringer-Mannheim Biochemicals (Indianapolis, IN). ADA was >90% pure by SDS-PAGE and was used to prepare Affigel affinity columns without further purification. Cell culture reagents were obtained from Gibco Laboratories (Grand Island, NY). Polyvinylidene difluoride membrane (PVDF, Immobilon-P) was obtained from Millipore (Bedford, MA). Nitrocellulose membrane was obtained from Schiecher & Schuell, Inc. (Keene, NH). [3H]Glucosamine was stored at -70°C. Normal human renal tissue was obtained at the time of surgery and was minced and then forced through a fine sieve in 50 mM Tris (pH 7.2). Aliquots of each fraction were analyzed by 7.5% SDS-PAGE under reducing conditions. Cells were silver stained to visualize protein. ADAbp-containing fractions were pooled. An aliquot was set aside for later purification by ADA-Affigel affinity matrix. The remainder of the ADAbp-containing fractions were precipitated by the addition of an equal volume of ice-cold acetone and stored overnight at -20°C. The precipitate was recovered by centrifugation and dissolved in sample buffer for SDS-PAGE. In parallel experiments, renal tissue was lysed and bound overnight to WGA-Sepharose. Bound glycoproteins were eluted with buffer containing 10% N-acetyl-glucosamine. Eluted material was incubated with mAb S27-Affigel and processed as above. An aliquot of ADAbp was further characterized by binding to ADA-Affigel. After binding overnight, the Affigel was washed with 30 vol of 50 mM Tris, pH 7.4, containing 0.1% NP-40, 0.5 M NaCl, and 1 mM EDTA, followed by low-salt buffer (150 mM NaCl). ADAbp was eluted with SDS-containing sample buffer and subjected to electrophoresis on a 7.5% polyacrylamide gel under reducing conditions. Proteins were visualized by silver stain.

Because renal tissue was not easily obtained, it was necessary to maximize the yield of ADAbp for use in amino acid analysis and sequence analysis of tryptic peptides. Material that did not bind to lectin under the stringent high-salt conditions described above was further purified by binding to mAb S27-Affigel affinity matrix and pooled with the ADAbp isolated above.

Amino Acid Analysis of ADAbp. An aliquot, representing 10% of the total mAb S27 affinity-purified ADAbp, was subjected to electrophoresis on a 0.75-mm thick 7.5% polyacrylamide gel under reducing conditions. ADAbp was electrotransferred onto Immobilon-P membrane in 10 mM 3-cyclohexylamino-1-propanesulfonic acid (CAPS) buffer, pH 11.0, containing 10% methanol (9). Transfer was complete after 75 min at 50 V (0.5 A). ADAbp was visualized by staining with 0.2% Ponceau S in 1% acetic acid. The ADAbp band was excised, destained in water, and allowed to air dry. Amino acid analysis was performed at the Harvard
was performed at the Harvard Microchemistry Facility. ADAbp, affinity purified with mAb S27, was separated by SDS-PAGE and electrotransferred onto nitrocellulose membrane. The band at 110 kDa (ADAbp) was excised. Subsequent processing was performed at the Harvard Microchemistry Facility. ADAbp was submitted to in situ digestion with trypsin (11), omitting the NaOH wash. The resulting peptide mixture was separated by narrow-bore HPLC using a Vydac C18 2.1 x 151Ymm reverse-phase column on a 1090 HPLC/1040 diode array detector (Hewlett-Packard Co., Palo Alto, CA). Optimum fractions from each peptide chromatogram were chosen based on differential UV absorbance at 210, 277, and 292 nm, peak symmetry, resolution, and predictive column retention. Automated Edman degradation was performed on a protein sequencer (477A; Applied Biosystems, Inc., Foster City, CA) by standard methods except that the reaction cartridge temperature was raised to 53°C during coupling with a commensurate decrease in R2 delivery and drydown time. Details of strategies for the selection of peptide fractions and their microsequencing have been previously described (12).

Demonstration of DPP IV Activity. Cells grown in culture were washed five times with PBS, then removed from the culture flasks with a rubber policeman and resuspended in PBS. An aliquot was diluted into trypan blue and counted on a hemocytometer. Cells were collected by centrifugation at 2,000 g for 10 min and lysed by incubation at 4°C for 30 min in 50 mM Tris containing 1% NP-40 and 20 μg/ml aprotinin. The volume of lysis buffer was adjusted so that the number of cells per microliter was equal for all samples. Lysates were incubated for 3 h with either 3.75 μg mAb (S27 or S4) or with no additions. A 20-μl pellet of RAMPS was then added to samples containing mAb and to control samples. Samples were incubated overnight at 4°C with rocking. RAMPS pellets were washed five times with cold 50 mM Tris, pH 7.4, containing 1% NP-40, then washed once with cold H2O. Antibody-antigen complexes were dissociated by rocking for 10 min at room temperature with 100 mM glycine buffer, pH 3.5, containing 0.1% NP-40. Samples were then subjected to electrophoresis on a 7.5% native polyacrylamide gel (SDS and β-mercaptoethanol omitted). Gels were stained for DPP IV activity by a modification of the method of Yoshimori and Walter (13). Gels were incubated at room temperature for 30 min in a solution of 0.5 mM glycyl-prolyl-4-methoxy-B-naphthylamide and 1.25 mg/ml tetrazotized o-dianisidine in 0.2 M Tris, pH 7.4. Positive staining was indicated by an insoluble precipitate formed by the cleaved 4-methoxy-B-naphthylmide and tetrazotized o-dianisidine. In other experiments, lyses also were incubated overnight at 4°C with 40-μl pellets of either ADA-Affigel, BSA-Affigel, or with no additions (ADA and BSA were coupled to Affigel-15 at 5-6 mg/ml of gel). Affigel pellets were washed and eluted as above. Samples were subjected to electrophoresis on a 7.5% native polyacrylamide gel and stained for DPP IV activity.

The ability of mAb S27 to bind to the same protein as that bound by ADA-Affigel was tested as follows. Lysates of SK-RC-28 renal carcinoma cells were incubated with either 20 μg of mAb S27 or with no additions for 3 h at 4°C. A 50-μl pellet of RAMPS was then added to each sample. Samples were incubated overnight at 4°C with rocking. The samples were centrifuged to pellet the RAMPS, and the supernatants were recovered. Supernatants from lyses incubated only with RAMPS were then incubated overnight with either ADA-Affigel or with BSA-Affigel. Supernatant that had been preincubated with mAb S27 and RAMPS was incubated overnight with ADA-Affigel. Proteins bound to the RAMPS or Affigel were dissociated with glycine buffer as described above.

Results

Complete Neoplastic Transformation of Melanocytes by v-Ha-ras In Vitro Induces Loss of ADAbp Expression. We have reported that transduction and expression of v-Ha-ras oncogene in cultured normal human diploid melanocytes can induce a subset of traits that distinguish melanoma from melanocytes (6, 8). Long-term expression of v-Ha-ras results in complete neoplastic transformation, with melanocytes acquiring all the phenotypic and genotypic characteristics observed in malignant melanoma in vivo (7). Transformation occurred in at least two phases and was associated with spontaneous chromosomal instability. We have found that extinction of ADAbp expression by v-Ha-ras expression in melanocytes occurred late in the transformation process, and correlated with a subset of traits that included PMA-independent growth, tumorigenicity in nu/nu mice, and the development and retention of specific chromosomal markers.

The v-Ha-ras oncogene was introduced into a passage 3 human foreskin melanocyte culture, designated 10W, by a murine retrovirus carrying the v-Ha-ras oncogene and pseudotyped with amphotropic murine leukemia virus (MuLV) 4070A. In the early phase of growth, 10W ras/early cells changed morphology, were hyperploid, and acquired anchor-age-independent growth, but remained dependent on the exogenous growth stimulator PMA for proliferation and survival (8). 10W ras/early cells continued to express ADAbp (Fig. 1, A and B), and the level of cell surface expression of ADAbp was similar to 10W parental cells (data not shown). At ~6 mo, foci of cells with distinct morphology appeared in the 10W ras/early culture. The 10W ras/late cell line, established from these foci, grew independently of exogenous growth factors in the culture medium and acquired specific

![Figure 1](image-url)
karyotypic abnormalities frequently detected in human melanomas (i.e., iso 6p, iso 9q, and del 1p) (7). 10W nas/late cells did not express ADAbp at the cell surface, and ADAbp synthesis was not detected by metabolic labeling (Fig. 1, A and B). Thus, in this in vitro model of sequential neoplastic transformation of melanocytes, loss of ADAbp expression corresponded to acquisition of independence from exogenous growth factors and was associated with specific chromosomal alterations.

**Purification of Human ADAbp.** ADAbp was purified from normal human tissue to determine its identity and possible function. Kidney is the most abundant source of ADAbp, and was used because of difficulty in obtaining large quantities of melanocytes for ADAbp purification. The structure of ADAbp derived from kidney and melanocytes cannot be distinguished: ADAbp from both cell types has identical molecular mass and expresses three distinct epitopes recognized by different mAbs against ADAbp (mAbs S6, S23, and S27) (6, 14, 15).

Initially, an NP-40 lysate was enriched for glycoproteins by lectin affinity purification. This fraction was then affinity purified using mAb S27, directed against ADAbp, coupled to an Affigel support matrix. Proteins eluted from the mAb S27 affinity column included a prominent band present at ~110 kD, corresponding to the known molecular mass of ADAbp (Fig. 2). A second band was present at 132 kD, and a broad region of staining was observed between 97 and 110 kD. Affinity-purified fractions were pooled and further enriched by a broad region of staining was observed between 97 and 110 kD. Affinity-purified fractions were pooled and further enriched by ADA-Affigel affinity matrix. The 110- and 97-110-kD bands were bound by ADA-Affigel (Fig. 2). Thus, the 110-kD band was characterized as a glycoprotein that bound to ADA-Affigel support matrix (left). Eluted material was subjected to 7.5% SDS-PAGE under reducing conditions. Proteins were visualized by silver stain. ADAbp is seen as a 110-kD band.

Partial breakdown products, intermediate forms, or alternatively processed forms of ADAbp. The 132-kD protein band that copurified with the 110-kD band on mAb S27-Affigel did not bind to ADA. We speculate that the 132-kD band is a distinct protein associated with ADAbp in the cell membrane, although it could represent an alternatively processed form of ADAbp that does not bind to ADA.

**Sequences of Tryptic Peptides of ADAbp and Amino Acid Analysis.** Five tryptic peptides (totaling 63 amino acids) were sequenced from the 110-kD band of ADAbp. Search of the EMBL/GenBank databases revealed 100% identity (63/63 amino acids) to human CD26, recently reported by Tanaka et al. (16). CD26 is also known as DPP IV (17). There was 87% amino acid homology (55/63 amino acids) to sequences within a cDNA-encoding DPP IV isolated from rat liver (Fig. 3) (18). Peptides corresponded to amino acid positions 259–267, 374–382, 493–522, 598–611, and 659–669 in the putative extracellular domain of human CD26/DPP IV, determined from the deduced polypeptide sequence of human ADAbp described by Tanaka et al. (16).

The 110-kD band was also analyzed for amino acid composition, deduced from the known molecular mass of the ADAbp core polypeptide (85 kD [5]) (Table 1). We compared the amino acid composition of human and rat DPP IV to human ADAbp, revealing nearly identical patterns (Table 1). The 97–110-kD region was also analyzed by excising a band at ~97 kD. The 97-kD band had an amino acid composition that was similar to that of the 110-kD form (Table 1), supporting the identity of core polypeptides for 97- and 110-kD forms. Variations in composition of individual amino acids could be attributed to protein yields. Derivatized amino acids from the 97-kD protein (from 0.2 μg) yielded peaks approximately threefold higher than background noise compared with data generated from higher yields for the 110-kD protein (1.7 μg). For example, glycine yielded a higher value.

---

**Figure 2.** Purification of ADAbp from normal human renal tissue. Lysates, enriched for glycoproteins by lectin affinity purification, were affinity purified using mAb S27 bound to an Affigel support matrix (left). Affinity-purified fractions were pooled and further enriched by binding to ADA-Affigel affinity matrix (right). Eluted material was subjected to 7.5% SDS-PAGE under reducing conditions. Proteins were visualized by silver stain. ADAbp is seen as a 110-kD band.

**Figure 3.** Amino acid sequence (three-letter code) of five human ADAbp peptides compared with human DPP IV and rat DPP IV. Identical amino acids are indicated by dashes.
in the 97- than 110-kD protein, presumably due to contamination from glycine in the SDS-PAGE buffer. Methionine, which is only moderately stable during acid hydrolysis, was completely destroyed in the 97-kD protein and >50% was destroyed in the 110-kD protein, based on comparison with derived human DPP IV amino acid sequence.

### Table 1. Comparison of Amino Acid Composition of Human ADAbp with That of Human 97-kD Band, Human DPP IV, and Rat DPP IV

|       | Human ADAbp | Human 97 kD | Human DPP IV | Rat DPP IV |
|-------|--------------|-------------|--------------|------------|
| ALA   | 46           | 37          | 40           | 47         |
| ARG   | 37           | 29          | 30           | 29         |
| ASN   | 40           | 34          |              |            |
| ASP   | 46           | 46          |              |            |
| ASX   | 113          | 95          |              |            |
| CYS   | ND           | ND          | 12           | 13         |
| GLN   | 30           | 33          |              |            |
| GLU   | 40           | 41          |              |            |
| GLX   | 82           | 84          |              |            |
| GLY   | 53           | 117         | 43           | 40         |
| HIS   | 23           | 13          | 19           | 21         |
| ILE   | 39           | 22          | 49           | 46         |
| LEU   | 57           | 38          | 62           | 63         |
| LYS   | 48           | 41          | 40           | 44         |
| MET   | 7            | 0           | 15           | 14         |
| PHE   | 28           | 18          | 31           | 31         |
| PRO   | 22           | 16          | 29           | 30         |
| SER   | 68           | 79          | 64           | 66         |
| THR   | 60           | 35          | 50           | 50         |
| TRP   | ND           | ND          | 21           | 21         |
| TYR   | 44           | 31          | 56           | 50         |
| VAL   | 47           | 27          | 49           | 48         |

### Discussion

ADAbp is a cell marker whose expression is not restricted to a specific lineage or particular stage of differentiation within a lineage. Rather, the regulation and function of ADAbp must be considered within frames of reference, depending on cell type. ADAbp was originally characterized as a surface mem-
brane glycoprotein expressed on a variety of secretory and absorptive epithelia (20, 21), fibroblasts (4), T lymphocytes (22), and melanocytes (6). Our interest in ADAbp has focused on its expression in normal melanocytes and its exquisite regulation during melanocyte transformation.

We have found that ADAbp is identical or analogous to CD26 and DPP IV, based on enzymatic characteristics and peptide sequences. In retrospect, the three markers, ADAbp, CD26, and DPP IV, are indistinguishable with regard to patterns of expression in tissues (23) and biochemical properties (e.g., masses of mature forms [5, 16, 18] and formation of dimers [24, 25]). Human ADAbp and CD26/DPP IV are 100% identical in five peptide regions ranging over approximately two-thirds of the CD26/DPP IV protein. Furthermore, ADAbp and CD26/DPP IV have core polypeptides with the same apparent mass (5, 16, 18), supporting the notion that ADAbp and CD26/DPP IV are encoded by the same gene, although this does not formally rule out the possibility that ADAbp could be encoded by a homologous gene. The derived amino acid sequence of human CD26 has recently been reported, and predicts a type II transmembrane molecule (16). CD26 cDNA encodes a potential signal sequence, followed by a large 738-amino acid extracellular domain (containing 10 possible Asn-linked glycosylation sites and a cysteine-rich region) on the carboxyl side of the putative transmembrane domain, and a short six-amino acid cytoplasmic domain (16). Of particular interest, the peptide sequence Gly-Trp-Ser-Tyr-Gly at amino acid positions 627-631 of human CD26 is a potential catalytic site for serine proteases and esterases (26).

ADAbp, DPP IV, and CD26 have each been characterized within the context of specific cell and tissue types. For instance, ADAbp is expressed on discrete subsets of epithelial cells in kidney tubules (21), and ADAbp in colon carcinoma cells is regulated according to the state of cellular differentiation (27). Within the T lymphocyte lineage, CD26 has been defined as a marker of activated T cells (16, 28). A list of potential functions is equally complex. ADAbp on epithelial cells has been defined by binding to ADA; DPP IV in liver, kidney, bowel, and other tissues has been detected by ectopeptidase activity, and CD26 on T cells plays a putative role in signal transduction (16) but also binds to collagen (29).

Within the melanocyte lineage, ADAbp expression is specifically extinguished during malignant transformation and tumor progression, without regard to stage of melanocyte differentiation (6, 7). ADAbp is a candidate for a gene product that is specifically downregulated or altered during neoplastic transformation. Thus, ADAbp could suppress the neoplastic phenotype in melanoma cells, based on the strong correlation between malignant phenotype and ADAbp regulation. This view fits a paradigm for neoplasia, where losses or defects of cell function have been implicated increasingly in the pathogenesis of many (if not most) human cancers. Gene products of this type stand in contrast to dominant acting oncogenes defined originally in tumor viruses. The tumor suppression phenomenon has been defined genetically by: (a) somatic cell hybrids or by transfer of chromosomes, parts of chromosomes, or specific genes into tumor cells (30, 31); (b) discovery of homozygous recessive mutations in certain hereditary cancers (e.g., Wilm's tumor [32] and retinoblastoma [33]); and (c) nonrandom deletions, alterations, or mutations at defined loci (e.g., p53 [34]) in human cancers, with presumed associated defects in gene function. Genetic approaches have not yet defined recessive oncogenes in human melanoma, although several candidate regions on chromosomes 1, 6, and 9 have been identified (35). Studies using somatic cell hybrids have tentatively assigned the genes encoding both ADAbp (36) and DPP IV (37) to chromosome 2, a chromosome that is altered in ~35% of melanoma specimens (35). Gross alterations in chromosome 2 probably occur during melanoma progression rather than as early events in melanocyte transformation, based on inconsistent alterations in chromosome 2 in different metastases within the same patient and the lack of chromosome 2 changes in potential precursor lesions of melanoma (35). The relationship between melanoma progression and alterations in chromosome 2 are consistent with our observations in vitro that loss of ADAbp expression is correlated with specific steps in melanoma progression. Notably, a specific subset of melanomas derived from the uvea of the eye have shown specific, nonrandom allelic losses in chromosome 2 (38).

We have been able to induce loss of ADAbp expression in melanocytes in vitro in two systems: by transfection with Ha-nas followed by long-term culture, and by repeated passage in the presence of isobutylmethylxanthine (6, 7). In both cases, downregulation of ADAbp expression coincided with the emergence of growth factor independence, while other traits characteristic of melanocyte transformation, such as anchorage-independent growth, loss of contact inhibition, induction of class II MHC antigens, and upregulation of GD3 ganglioside, did not correlate with the loss of ADAbp expression. These observations suggest that ADAbp might function to regulate cell growth, perhaps through the degradation of crucial growth factors. This proposed function for ADAbp on melanocytes is based on the observed biological properties of DPP IV, which hydrolyzes the carboxy-terminal side of dipeptide sequences, with a preference for proline at the second residue but also recognizing alanine at a lower efficiency. DPP IV is the primary enzyme responsible for cleavage and inactivation of serum growth hormone-releasing hormone (39). Potential cleavage sites exist in the matrix component collagen and the cytokines IL-1β, IL-2, and GM-CSF (28). Interestingly, a possible cleavage site also exists in ADA, which has an amino-terminal Met-Ala (40).

A model relevant to DPP IV expression on melanocytic cells has been proposed for another cell surface peptidase, CD10/neutral endopeptidase 24.11, in small cell lung cancer (SCLC). CD10 is a metalloendopeptidase that hydrolyzes a number of natural peptides, including atrial natriuretic factor, angiotensins 1 and 2, substance P, endothelin, bradykinin, oxytocin, and Leu- and Met-enkephalins (41). CD10, which is expressed at low levels on SCLC, cleaves potential autocrine growth factors for SCLC, including bombesin- and gastrin-releasing peptide (42). CD10 can inhibit proliferation...
of growth factor–dependent SCLC cells, and inhibition of CD10 activity can reverse this block (42).

An interesting potential substrate for DPP IV is basic fibroblast growth factor (bFGF), based on reported amino-terminal peptide sequences (43). Although there is no evidence that DPP IV cleaves bFGF, at least one form of purified bFGF has an amino-terminal sequence (i.e., Pro-Ala-Leu-Pro) that provides a substrate for two successive rounds of proteolysis by DPP IV. Basic FGF fulfills criteria as an autocrine growth factor for melanoma: (a) exogenous bFGF can stimulate the growth of melanocytes in vitro (44); (b) melanoma cells express both bFGF and its receptor (45-47); (c) antibodies against bFGF have been used to inhibit melanoma cell growth in vitro (48); (d) transfection of melanoma cells with antisense oligonucleotides against bFGF can inhibit melanoma proliferation (49); and (e) melanocytes transfected with bFGF cDNA grow independently of exogenous growth factors, although they form only benign, not invasive, tumors when injected into nude mice (50). Thus, expression of bFGF alone is insufficient to confer a full malignant phenotype, but rather appears to represent a critical step in a sequence necessary for full neoplastic transformation. In the 10W melanocyte system, bFGF transcripts were detected in both 10W ras/early and 10W ras/late cells, but not parental 10W cells (7). Interestingly, both ADAbp and CD10 were expressed by parental 10W cultures and by 10W ras/early cells, which maintained only a partially transformed phenotype and required exogenous growth factors for survival. However, expression of both peptidases was extinguished in the fully transformed 10W ras/late cell strain (7).

We thank William S. Lane at the Harvard Microchemistry Facility for amino acid analysis and sequencing of tryptic peptides. We also thank Drs. David Nanus and Neil Bander for assistance in isolation of ADAbp.

This research was supported by the Louis and Anne Abrons Foundation and grant CA-37907 from the National Cancer Institute. M. E. Morrison has been supported by the Charles A. Dana Foundation, the Norman and Rosita Winston Foundation, and training grant CA-09512 from the National Cancer Institute.

Address correspondence to Mark E. Morrison, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

Received for publication 12 November 1992.

References

1. Clark, W.H.J., D.E. Elder, D. Guerry IV, M.N. Epstein, M.H. Greene, and M. van Horn. 1984. A study of tumor progression: the precursor lesions of superficial spreading and nodular melanoma. Hum. Pathol. 15:1147.
2. Herlyn, M., and H. Koprowski. 1988. Melanoma antigens: immunological and biological characterization and clinical significance. Annu. Rev. Immunol. 6:283.
3. Lynch, S.A., B. Bouchard, S. Vijayasaradhi, H. Yuasa, and A.N. Houghton. 1991. Antigens of melanocytes and melanoma. Cancer Metastasis Rev. 10:141.
4. Andy, R.J., and R. Kornfeld. 1982. The adenosine deaminase binding protein of human skin fibroblasts is located on the cell surface. J. Biol. Chem. 257:7922.
5. Andy, R.J., and R. Kornfeld. 1984. Biosynthesis of the adenosine deaminase-binding protein in human fibroblasts and hepatoma cells. J. Biol. Chem. 259:9832.
6. Houghton, A.N., A.P. Albino, C. Cordon-Cardo, L.J. Davis, and M. Eisinger. 1988. Cell surface antigens of human melanocytes and melanoma. Expression of adenosine deaminase binding protein is extinguished with melanocyte transformation. J. Exp. Med. 167:197.
7. Albino, A.P., G. Sozzi, D.M. Nanus, S.C. Jhanwar, and A.N. Houghton. 1992. Malignant transformation of human melanocytes: induction of a complete melanoma phenotype and genotype. Oncogene. 7:2315.
8. Albino, A.P., A.N. Houghton, M. Eisinger, J.S. Lee, R.R.S. Kantor, A.I. Oliff, and L.J. Old. 1986. Class II histocompatibility antigen expression in human melanocytes transformed by Harvey murine sarcoma virus (Ha-MSV) and Kirsten MSV retroviruses. J. Exp. Med. 164:1710.
9. Matsudaira, P. 1987. Sequence of picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes. J. Biol. Chem. 262:10035.
10. Ebert, R.F. 1986. Amino acid analysis by HPLC: optimized conditions for chromatography of phenylthiocarbamyl derivatives. Anal. Biochem. 154:431.
11. Aebersold, R.H., J. Leavitt, R.A. Saavedra, L.E. Hood, and J. Biol. Chem. 262:10035.
12. Lane, W.S., A. Galat, M.W. Harding, and S.L. Schreiber. 1991. Complete amino acid sequence of the FK506 and rapamycin binding protein, FRBP, isolated from calf thymus. J. Protein. Chem. 10:151.
13. Yoshimoto, T., and R. Walter. 1977. Post-proline dipeptidyl aminopeptidase (dipeptidyl aminopeptidase IV) from lamb kidney. Purification and some enzymatic properties. Biochim. Biophys. Acta. 485:301.
14. Ueda, R., S.I. Ogata, D.M. Morrissey, C.L. Finstad, J. Szkuiack, W.F. Whitmore, H.F. Oettgen, K.O. Lloyd, and L.J. Old. 1981. Cell surface antigens of human renal cancer defined by mouse monoclonal antibodies: identification of tissue-specific kidney glycoproteins. Proc. Natl. Acad. Sci. USA. 78:5122.
15. Andy, R.J., C.L. Finstad, L.J. Old, K.O. Lloyd, and R. Kornfeld. 1984. The antigen identified by a mouse monoclonal antibody raised against human renal cancer cells is the adenosine deaminase binding protein. J. Biol. Chem. 259:12844.

16. Tanaka, T., D. Camerini, B. Seed, Y. Torimoto, N.H. Dang, J. Kameoka, H.N. Dahlberg, S.F. Schlossman, and C. Morimoto. 1992. Cloning and functional expression of the T cell activation antigen CD26. J. Immunol. 149:481.

17. Ulmer, A.J., T. Mattern, A.C. Feller, E. Heymann, and H.D. Nelson. 1981. Specific chromosome loss associated with the expression of tumorigenicity in human cell hybrids. Somatic Cell Genet. 7:699.

18. Ogata, S., Y. Misumi, and Y. Ikehara. 1989. Primary structure of rat liver dipeptidyl peptidase IV deduced from its cDNA and identification of the NH2-terminal signal sequence as the membrane-anchoring domain. J. Biol. Chem. 264:3596.

19. Schrader, W.P., and C.A. West. 1985. Adenosine deaminase complexing proteins are localized in exocrine glands of the rabbit. J. Histochem. Cytochem. 33:508.

20. Daddona, P.E., and W.N. Kelley. 1978. Human adenosine deaminase complexing protein (ADCP) gene(s) to human chromosome 2 in rodent-human somatic cell hybrids. Hum. Genet. 59:317.

21. Dinjens, W.N.M., J. ten Kate, E.P. van der Linden, J.T. Wijnen, J.P. Kettner, and W.W. Bachovchin. 1991. Inhibition of dipeptidyl peptidase IV and tryptsin-like enzymatic degration of human growth hormone-releasing hormone in plasma. J. Clin. Invest. 83:1533.

22. Daddona, P.E., D.S. Shewach, W.N. Kelley, P. Argos, A.F. Markham, and S.H. Orkin. 1984. Human adenosine deaminase, cDNA and complete primary amino acid sequence. J. Biol. Chem. 259:1201.

23. Shipp, M.A., J. Vijayaraghavan, E.V. Schmidt, E.L. Castetter, L. D'Amato, L.B. Hersh, and E.L. Reinherz. 1989. Common acute lymphoblastic antigen (CALLA) is active neutral endopeptidase 24.11 ("enkephalinase"): direct evidence by cDNA transfection analysis. Proc. Natl. Acad. Sci. USA. 86:297.

24. Abraham, J.A., J.L. Whang, A. Tumolo, A. Mergia, J. Friedman, D. Gospodarowicz, and J.C. Fiddes. 1986. Human basic fibroblast growth factor: nucleotide sequence and genomic organization. EMBO (Eur. Mol. Biol. Organ.) J. 5:2523.

25. Halaban, R., S. Ghosh, and A. Baird. 1987. bFGF is the putative natural growth factor for human melanocytes. In Vitro Cell. & Dev. Biol. 23:47.

26. Albino, A.P., B.M. Davis, and D.M. Nanus. 1991. Induction of growth factor RNA expression in human malignant melanoma: markers of transformation. Cancer Res. 51:4815.

27. Halaban, R. 1991. Growth factors and tyrosine protein kinases in normal and malignant melanocytes. Cancer Metastasis Rev. 10:129.

28. Rodeck, U., K. Melber, R. Kath, H.D. Menssen, M. Varello, B. Atkinson, and M. Herlyn. 1991. Constitutive expression of multiple growth factor genes by melanoma cells but not normal melanocytes. J. Invest. Dermatol. 97:20.

29. Halaban, R., B.S. Kwon, S. Ghosh, P.D. Bovi, and A. Baird. 1988. bFGF as an autocrine growth factor for human melano-
nomas. Oncogene Res. 3:177.
49. Becker, D., C.B. Meier, and M. Herlyn. 1989. Proliferation of human malignant melanomas is inhibited by antisense oligodeoxynucleotides targeted against basic fibroblast growth factor. EMBO (Eur. Mol. Biol. Organ.) J. 8:3685.

50. Dotto, G.P., G. Moellmann, S. Ghosh, M. Edwards, and R. Halaban. 1989. Transformation of murine melanocytes by basic fibroblast growth factor cDNA and oncogenes and selective suppression of the transformed phenotype in a reconstituted cutaneous environment. J. Cell. Biol. 109:3115.