[CANCER RESEARCH 61, 7642–7646, October 15, 2001]

HDM2 Protein Overexpression, but not Gene Amplification, is Related to Tumorigenesis of Cutaneous Melanoma[1]

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ABSTRACT

We investigated the role of alterations of *HDM2*, the human homologue of murine *mdm2*, in the tumorigenesis and progression of cutaneous melanoma. A well-characterized cohort of 172 cases representing different points in the spectrum of melanocyte transformation (16 dysplastic nevi, 11 melanomas *in situ*, 107 invasive primaries, and 38 metastatic lesions), as well as 11 human melanoma cell lines were examined by immunohistochemistry and Western blotting for HDM2 protein expression, and by either Southern blotting (SB) or fluorescence *in situ* hybridization for *HDM2* gene amplification. HDM2 overexpression, defined as >20% tumor cells showing nuclear immunoreactivity, was observed in 1 of 16 (6%) dysplastic nevi, 3 of 11 (27%) melanomas *in situ*, and 81 of 145 (56%) invasive primary and metastatic melanomas. Comparable frequencies of HDM2 overexpression were observed among invasive primary cases with differing tumor thicknesses as well as among the metastatic cases: 21 of 40 (53%) at ≤1.5 mm; 31 of 50 (62%) at 1.6–3.9 mm; 10 of 17 (58%) at >4 mm; and 19 of 38 (50%) metastases. *HDM2* amplification was observed in 1 of 88 (1%) primary cases using fluorescence *in situ* hybridization, and in 0 of 12 (0%) metastatic cases that overexpressed HDM2 using SB. Melanoma cell lines expressed HDM2 protein, but there was no evidence of amplification by SB. Our data suggest that HDM2 protein overexpression is common in invasive and metastatic melanoma. Observing HDM2 overexpression in noninvasive melanoma suggests that expression of this oncogene may play an early role in melanocyte transformation. *HDM2* amplification occurs infrequently, and other mechanisms that up-regulate HDM2 expression are under investigation.

INTRODUCTION

Studies of alterations in cell cycle control pathways that occur in the early stages of melanoma tumor progression have focused mainly on the tumor suppressors *p16INK4A* and *TP53*, which are uncommonly mutated in sporadic melanomas (Reviewed in Refs. 1 and 2). Less is known about the role of oncogenes in melanoma (Reviewed in Ref. 3).

The oncogene *HDM2* is a critical, negative regulator of the *p53* pathway. *HDM2* maps to chromosome 12q13 and encodes a M_r 90,000 zinc finger protein (HDM2) that functions as a ubiquitin E3 ligase. HDM2 binds to the transcriptional activation domain of p53, blocking its function, and via ubiquitination, targets p53 for proteosome-mediated enzymatic degradation (Reviewed in Ref. 4).

An oncogenic role for deregulated *HDM2* expression is supported by both *in vitro* and *in vivo* data. *In vitro*, the overexpression of *mdm2* in murine cells increases their growth rate (5). Transgenic mice overexpressing *mdm2* were tumor-prone, with 50% developing lymphomas and sarcomas. In comparison with *p53*-null mice, these mice showed an increased incidence of sarcomas that was maintained in crosses onto the *p53*-null background, demonstrating a p53-independent role for *HDM2* in tumorigenesis (6). In humans, *HDM2* is amplified in a variety of tumors (7), and HDM2 protein overexpression without amplification has also been observed in certain cancers (8, 9). Of special interest are recent reports that described enhancement of drug-induced apoptosis by antisense oligodeoxynucleotides and synthetic peptides targeted against HDM2 (10–12). These novel antitumor agents that stabilized p53 *in vitro*, and possess antitumor activity in xenograft models, may be appropriate agents for therapeutic interventions in patients whose tumors overexpress HDM2.

In the present study, we analyzed a well-characterized cohort of cases representing different stages of melanocyte transformation, as well as a group of human melanoma cell lines, for alterations affecting *HDM2* and the expression of its protein product to determine its possible role in melanoma tumorigenesis.

MATERIALS AND METHODS

Tumor Specimens and Cell Lines. A cohort of 172 cases including 16 dysplastic nevi, 11 melanomas *in situ*, 107 invasive cutaneous primaries, and 38 metastatic lesions was evaluated. Eighty-eight of the invasive primary melanomas were retrieved from the archives of the Dermatopathology Section of the University of California, San Francisco (San Francisco, CA). These cases were provided as tissue microarray for both FISH and IHC studies. Eighty-four cases (16 dysplastic nevi, 11 melanomas *in situ*, 19 invasive primaries, and 38 metastatic lesions) were obtained from the Memorial Sloan-Kettering Cancer Center archival bank. Representative H&E-stained sections of each specimen were examined microscopically to confirm the presence of melanoma as well as to evaluate thickness of the primary tumors analyzed. Primary invasive melanoma cases were grouped into one of three categories based on the thickness of the primary lesion according to American Joint Committee on Cancer staging criteria: thin, ≤1.5 mm; intermediate, 1.6–3.9 mm; and thick, >4 mm (13). Human metastatic melanoma cell lines (SK-MEL-19, -29, -85, -94, -100, -103, -147, -173, -187, -197, and -209) and the osteosarcoma cell line SJSA-1, which contains a known amplification at *HDM2*, were maintained in culture using RPMI 1640 with 10% FCS and 1% penicillin/streptomycin. For IHC experiments, cells were trypsinized, washed in PBS, and collected onto glass slides using a Shandon Cytospin centrifuge.

Assembly of Tissue Microarrays. The tissue microarray with 88 cases was constructed according to Kononen *et al.* (14). In brief, a tissue-arraying instrument (Beecher Instruments, Silver Spring, MD) was used to punch 0.6-mm biopsy cores of the most cellular areas of the tumors. The biopsy cores were arrayed in the recipient paraffin block according to the manufacturer's instructions. Multiple sections of 6-mm thickness were cut with a microtome using an adhesive-coated tape sectioning system (Instrumedics, Hackensack, NJ). H&E sections were used for the histological examination of the biopsy cores. Only cases with at least one area with a cohesive population of neoplastic melanocytes were included in the analysis.

FISH Analysis. FISH was performed on formalin-fixed tissue microarray sections representing 88 primary cases. Dual-color FISH was carried out on tissue sections of the array as described previously (15). Genomic clones containing the *HDM2* gene were provided by Vysis, Inc., Downers Grove, IL. A reference probe for the chromosome 12p (clone RMC12B3042A) was

Received 3/6/01; accepted 8/20/01.

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[1] Supported in part by NIH Grant K08 AR02129 (to D. P.) and by the Marvin and Roma Auerback Melanoma Fund (to B. C. B.). Also supported in part by the use of facilities at the Manhattan Veterans Affairs Medical Center, New York, NY.

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[3] The abbreviations used are: FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry.

Table 1 *HDM2 immunohistochemistry results*

	No. of positive cases	% of positive cases
Dysplastic nevi	1/16	6%
Melanomas *in situ*	3/11	27%
Primary invasive melanoma		
≤1.5 mm	21/40	53%
1.6–3.9 mm	29/48	60%
≥4.0 mm	12/19	63%
Metastatic melanoma	19/38	50%

obtained from the laboratory's resource. Probes were labeled by nick-translation with SpectrumOrange (Vysis, Inc.) or with digoxigenin (Boehringer Mannheim, Indianapolis, IN). Tissue sections were deparaffinized, hydrated, and pretreated for 2–4 min in 1 M sodium thiocyanate at 80°C, in 4 mg/ml pepsin in 0.2 M HCl at 37°C for 4–8 min. After dehydration, sections were denatured in 70% formamide and 2× SSC (pH 7.0) for 5 min at 72°C and hybridized over 48–72 h at 37°C in 10 μl of hybridization buffer containing 12.5–50 ng of labeled probes [50% formamide, 10% dextran sulfate, and 2× SSC (pH 7.0), and 20 μg of Cot-1 DNA; Life Technologies, Inc., Gaithersburg, MO]. Slides were washed three times in washing solution [50% formamide in 2× SSC (pH 7.0)] at 45°C, once in 2× SSC at 45°C, once in 2× SSC at room temperature, and once in 0.1% Triton X-100 in 4× SSC/at room temperature. Subsequently, sections were incubated with 10% BSA in 4× SSC in a moist chamber at 37°C and then with a FITC-labeled antidigoxigenin antibody (Boehringer Mannheim, Indianapolis, IN) diluted in 4× SSC with 10%. Sections were counterstained with 4,6-diamino-2-phenylindole (Sigma Chemical Co., St. Louis, MO) in an antifade solution. FISH signals were scored with a fluorescence microscope from Zeiss (Jena, Germany) using a ×63 objective. Criteria for amplification were: at least 2.5 times more test probe signals than reference signals in at least 30% of the tumor cells (16).

IHC. IHC was performed on all 172 cases and 11 cell lines. The primary antibody, used at a 1:500 dilution, was the well-characterized anti-HDM2 mouse monoclonal antibody, clone 2A10 (a gift of Dr. Arnold Levine, Rockefeller University, New York, NY; Refs. 17–19). IHC was performed using an avidin-biotin-peroxidase method and antigen retrieval. Sections were immersed in boiling 0.01 M citric acid (pH 6.0) for 15 min under microwave treatment to enhance epitope exposure. After cooling to room temperature, slides were incubated with the primary antibody overnight at 4°C. For negative controls the primary antibody was omitted. Biotinylated horse antimouse IgG antibodies (Vector Laboratories, Burlingame, CA; 1:500 dilution) were used as secondary reagents, applied for an incubation period of 1 h before avidin-biotin peroxidase complexes (Vector Laboratories; 1:25 dilution) and incubation for 30 min. Diaminobenzidine was used as the final chromogen, and hematoxylin was used as the nuclear counterstain. Nuclear immunoreactivities for HDM2 were classified into two categories defined as follows: (*a*) negative, <20% tumor cells displaying nuclear immunostaining; and (*b*) positive, >20% tumor cells with nuclear immunostaining. The cutoff point was based on our previous reports showing a correlation between HDM2 overexpression in >20% of tumor cells with worse clinicopathological parameters and clinical outcome in bladder, prostate, and laryngeal carcinomas (20–22).

Western Blotting. Cells were washed with cold PBS then lysed with an ice-cold buffer (pH 7.0) containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 400 mM NaCl, 10% glycerol, 0.5% NP40, and protease and phosphatase inhibitors. Lysates were placed on ice for 20 min before clarification by centrifugation. Protein determinations were performed using the Bradford method (Bio-Rad Laboratories, Hercules, CA). Twenty-five to 50 μg of each sample was fractionated by SDS-PAGE and transferred to Immobilon-P membranes (Millipore, Bedford, MA). Membranes were blocked with 5% nonfat dry milk, 0.1% Tween 20 in PBS, and probed with the anti-HDM2 mouse monoclonal antibody SMP-14 (Santa Cruz Biotechnology, Santa Cruz, CA). Protein loading was confirmed using an anti-Ran monoclonal antibody (Santa Cruz Biotechnology). Bands were visualized using horseradish peroxidase-conjugated antimouse secondary antibodies (Santa Cruz Biotechnology) and the SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL).

Southern Blotting. Genomic DNA was extracted from melanoma cell lines and from multiple 30-micron sections of snap-frozen, OCT-embedded, metastatic melanoma tissues that overexpressed HDM2 by IHC. DNA was

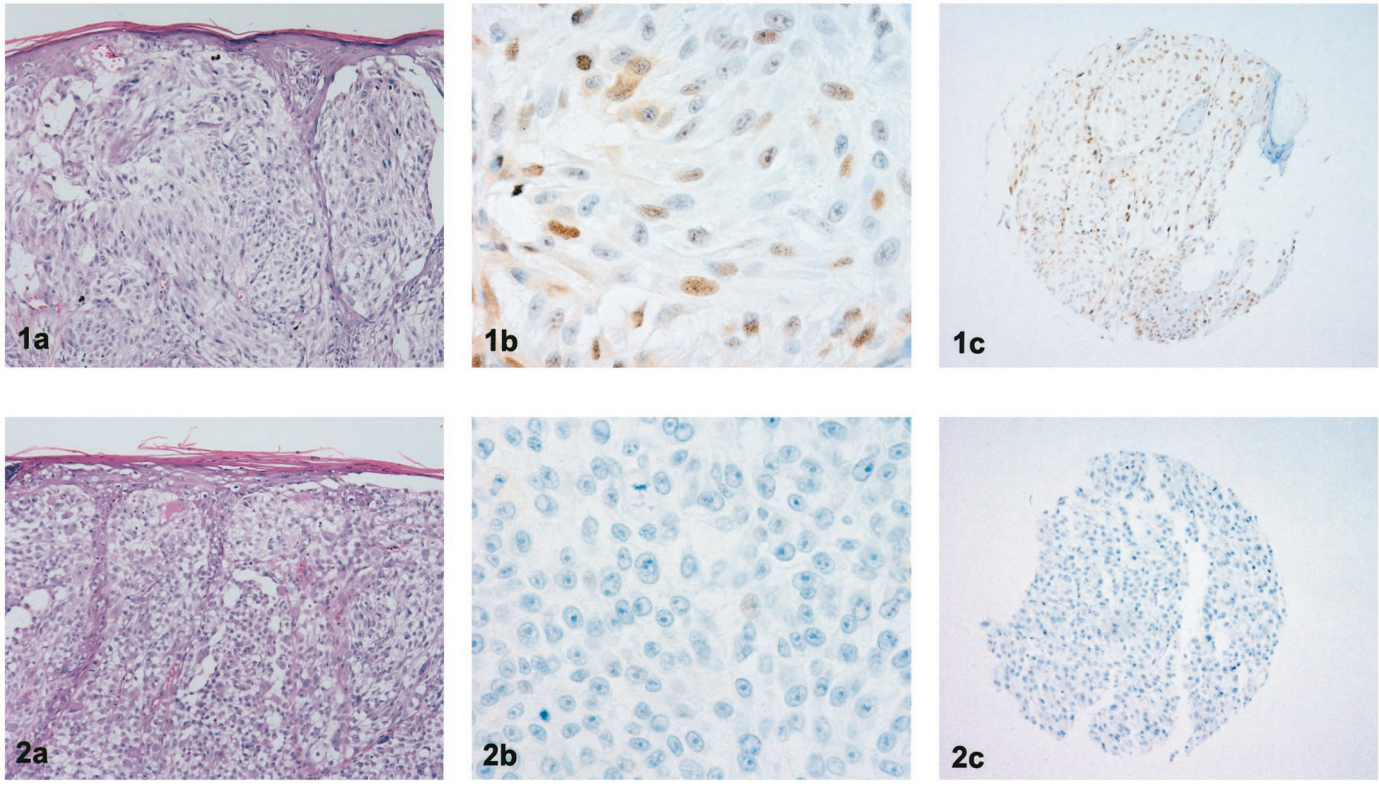

Fig. 1. Photomicrographs of two primary melanomas analyzed by IHC using the mouse monoclonal antibody 2A10 (anti-HDM2). *Top panel*: tumor 1, 1.5 mm thick. *1a*, full section stained with H&E, ×100; *1b*, full section stained with 2A10, ×400; *1c*, corresponding core section analyzed on the tissue array using 2A10, ×100. *Lower panel*: tumor 2, 3.2 mm thick. *2a*, full section stained with H&E, ×100; *2b*, full section stained with 2A10, ×400; *2c*, corresponding core section analyzed on the tissue array using 2A10, ×100. Note the similar immunophenotypes in the whole sections and in the representative core sections.

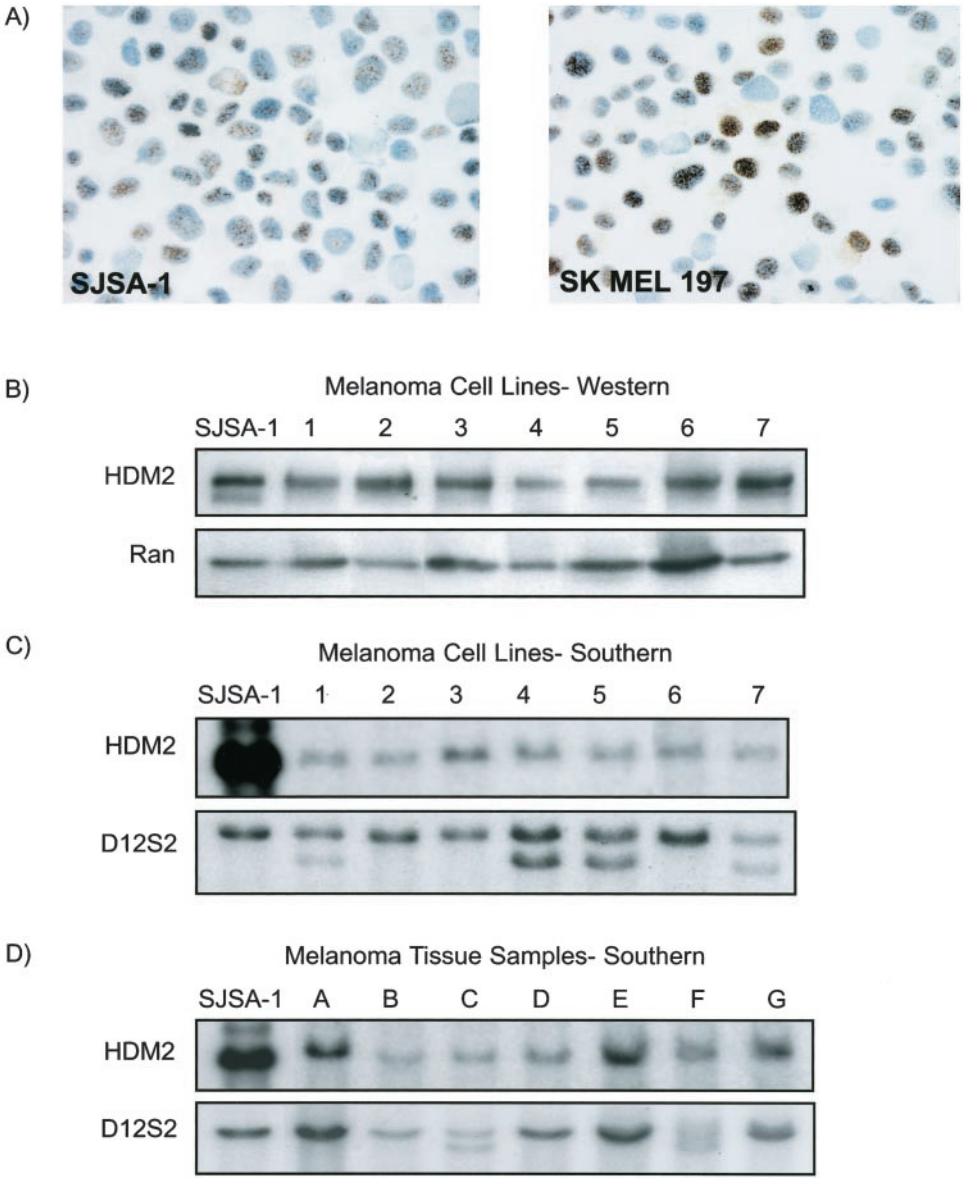

Fig. 2. *A*, IHC of cell lines using 2A10. *Left*, positive control osteosarcoma cell line, SJSA-1; *right*, melanoma cell line SK-MEL-197. *B*, Western blot analysis for HDM2 protein expression. Lysates from SJSA-1 and representative melanoma cell lines (*Lanes 1–7*) were probed using the anti-HDM2 mouse monoclonal antibody SMP-14. The anti-Ran mouse monoclonal antibody was used to verify loading. *Lane 1*, SK-MEL-19; *Lane 2*, SK-MEL-85; *Lane 3*, SK-MEL-94; *Lane 4*, SK-MEL-103; *Lane 5*, SK-MEL-147; *Lane 6*, SK-MEL-197; *Lane 7*, SK-MEL-209. *C*, Southern blot analysis of the same seven melanoma cell lines listed in *B*. *Left lane*, DNA from SJSA-1, known to have at least a 15-fold amplification of HDM2. Membranes were probed using a human cDNA probe against HDM2, stripped, and reprobed using the chromosome 12 probe D12S2 to verify loading. *D*, Southern blot analysis of seven metastatic melanoma tissues that overexpressed HDM2 by IHC. Note the lack of amplification in both the melanoma cell lines and metastatic tumors that overexpressed HDM2.

extracted using the Qiagen Tissue kit (Qiagen, Valencia, CA) digested with *Eco*RI, fractionated on 0.7% agarose gels, photographed, transferred to nylon membranes, and probed with a 1.6-kb fragment of *HDM2* cDNA (kindly provided by Dr. Arnold Levine, Rockefeller University, New York, NY) labeled with ^{32}P (Amersham Pharmacia Biotech, Piscataway, NJ) by random priming (Amersham, Amersham, United Kingdom). Membranes were washed and subjected to autoradiography at −70°C. To control for variations in sample loading and nonspecific chromosome-12 amplifications, membranes were stripped and reprobed with D12S2 (American Type Culture Collection, Rockville, MD), a marker that maps to chromosome 12, on the arm opposite of *HDM2*. DNA from the osteosarcoma cell line SJSA-1, known to have at least a 15-fold amplification of HDM2, was included on all membranes as a positive control (23).

RESULTS AND DISCUSSION

HDM2 nuclear immunoreactivities were observed in 1 of 16 (6%) dysplastic nevi, 3 of 11(27%) melanomas *in situ*, 81 of 145 (56%) melanoma cases, and 8 of 11 (73%) melanoma cell lines. Comparable frequencies of immunoreactivity were observed among primary invasive lesions of differing thicknesses and between primary lesions and metastases (Table 1). In normal surrounding tissues, HDM2 expression was rarely detected. These data suggest that HDM2 overexpression may be a relatively early event, and that this alteration is conserved during the progression from primary to metastatic disease.

Eighty-eight of the melanoma cases were assayed as a tissue microarray, with 0.6-mm cores present on a single microslide; the remaining 84 tissues were assayed conventionally, using one specimen/slide. To validate our ability to obtain representative IHC scores from the tissue microarray, we compared HDM2 expression in the core biopsies represented in the array with expression in corresponding whole sections in 20 randomly selected lesions. The results were similar in 18 of 20 (90%) cases, showing that potential heterogeneous HDM2 expression is unlikely to bias the data from the tissue microarrays (Fig. 1). In this context, two recent publications (by our group and others) have validated the use of tissue microarrays for expression profiling by IHC with high concordance rates (24, 25).

Applying IHC with the 2A10 monoclonal antibody to cytospin preparations of 11 melanoma cell lines, 8 lines (SK-MEL-19, -85, -94, -103, -147, -173, -197, and -209) showed moderate to intense nuclear staining; whereas the remaining 3 lines (SK-MEL-29, -187, and -192) showed either undetectable or weak HDM2 nuclear immunoreactivity.

Using Western blotting with a different anti-HDM2 monoclonal antibody, SMP-14, we observed the expected M_r 90,000 band in 10 of 11 cell lines. Representative cases are shown in Fig. 2. The cell line SK-MEL-187 was negative.

Amplification of *HDM2* is a commonly described mechanism leading to HDM2 overexpression. To investigate this mechanism, FISH analysis of 88 primary cases on the tissue microarray and Southern blotting on 11 melanoma cell lines and 12 metastatic melanomas that overexpressed HDM2 was performed. HDM2 amplification was observed in 1 of 88 (1%) primary cases and in none of the metastatic cases or cell lines (Fig. 2). The finding of infrequent *HDM2* amplifications is in agreement with two studies that used comparative genomic hybridization to show that amplifications encompassing the *HDM2* locus at chromosome 12q13 are an infrequent event in melanoma (26, 27).

Studies of alterations in cell cycle control pathways that occur in the early stages of melanoma tumor progression have focused mainly on the tumor suppressors *p16INK4A*, because of the presence of germ-line mutations in subsets of familial melanoma patients (1), and *TP53*, which is rarely mutated in the majority of melanomas (2, 28). Our previous analysis of p53 alterations in 57 cases from this cohort revealed that p53 protein accumulation and *TP53* mutations are late events in melanoma tumorigenesis. They were not seen in primary melanomas <4.0 mm thick, and they were found uncommonly (25%) in thick primary lesions (>4 mm) and in metastatic lesions (29).

The role of HDM2 overexpression in human melanoma tissues has been addressed in smaller cohorts by others (30–33). Our study is in agreement with that of Gelsleichter *et al.* (32), who also found overexpression in 56% of mostly metastatic cases, with little difference in positivity rates between primary and metastatic lesions. Our study examined a greater number of primary cases (107 *versus* 36) and investigated a mechanism driving HDM2 overexpression. Two studies that suggest increasing HDM2 expression with disease progression (31, 33) are limited by a relatively small number of metastatic cases, specifically 12 and 5 cases, respectively, compared with our study (n = 38). Other features of these studies that might contribute to the differences in results include a lack of cutoff points used to define HDM2 overexpression, generally low rates of immunopositivity, and other differences in methodology (*e.g.*, different antibodies).

Our data demonstrating overexpression of HDM2 in both *in situ* and thin invasive melanomas support the hypothesis that overexpression of HDM2 is an early event in melanoma tumorigenesis. Similar conclusions have been reported in several other tumor types. For example, in primary non-small cell lung cancer HDM2 was overexpressed in 70% of cases, with comparable rates of overexpression in early *versus* late clinical stages (34). In another study of squamous cell carcinoma of the oral mucosa, HDM2 overexpression was observed in dysplastic lesions that progressed to invasive disease (35). A similar observation was reported in colon cancer, where HDM2 expression patterns were similar in adenomas and colorectal carcinomas (36).

Overexpression of HDM2 protein in the absence of gene amplification has also been observed in other systems, such as Burkitt's lymphoma and breast cancer (37, 38). In addition, HDM2 overexpression, rather than amplification, was associated with a worse clinical outcome in soft tissue sarcomas (18). *In vitro*, translation enhancement of *HDM2* has been described in several tumor cell lines, including the melanoma cell line SK-MEL-2 (39). Increased HDM2 protein levels have also been observed in fibroblasts after treatment with basic fibroblast growth factor (40), or in various cell types as a result of signaling via the Ras/Raf/extracellular signal-regulated kinase kinase pathway (41). Interestingly, autocrine secretion of growth factors such as basic fibroblast growth factor may be an early event in melanocyte transformation (reviewed in Ref. 42) and may contribute to HDM2 overexpression in some cases.

Metastatic melanoma is a highly chemoresistant tumor (43). In this clinical setting, HDM2 overexpression is of particular interest, because recent studies have showed its correlation with a lack of response to chemotherapy (44, 45). Moreover, enhancement of drug-induced apoptosis by antisense oligodeoxynucleotides and synthetic peptides targeted against HDM2 has recently been described (10–12). These novel antitumor agents that stabilized p53 *in vitro* and possess antitumor activity in xenograft models may be effective therapeutic interventions in patients whose tumors overexpress HDM2.

In summary, we conclude that HDM2 overexpression is an early and frequent event in melanoma tumorigenesis. Because of the lack of *HDM2* gene amplification, we are currently investigating other mechanisms driving HDM2 overexpression.

ACKNOWLEDGMENTS

We thank Maria Dudas, Man Yee Lui, and Weiming Gai for technical assistance and Dr. Charles J. DiComo for review of the manuscript and many helpful discussions.

REFERENCES

1. Haluska, F. G., and Hodi, F. S. Molecular genetics of familial cutaneous melanoma. J. Clin. Oncol., *16:* 670–682, 1998.
2. Albino, A. P., Vidal, M. J., McNutt, N. S., Shea, C. R., Prieto, V. G., Nanus, D. M., Palmer, J. M., and Hayward, N. K. Mutation and expression of the *p53* gene in human malignant melanoma. Melanoma Res., *4:* 35–45, 1994.
3. Polsky, D., Cordon-Cardo, C., and Houghton, A. N. Molecular Biology of Melanoma. *In:* J. Mendelsohn, P. M. Howley, M. A. Israel, and L. A. Liotta (eds.), The Molecular Basis of Cancer, Ed. 2, pp. 385–404. Philadelphia: W. B. Saunders, 2001.
4. Piette, J., Neel, H., and Marechal, V. Mdm2: keeping p53 under control. Oncogene, *15:* 1001–1010, 1997.
5. Fakharzadeh, S. S., Trusko, S. P., and George, D. L. Tumorigenic potential associated with enhanced expression of a gene that is amplified in a mouse tumor cell line. EMBO J., *10:* 1565–1569, 1991.
6. Jones, S. N., Hancock, A. R., Vogel, H., Donehower, L. A., and Bradley, A. Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. Proc. Natl. Acad. Sci. USA, *95:* 15608–15612, 1998.
7. Momand, J., Jung, D., Wilczynski, S., and Niland, J. The *MDM2* gene amplification database. Nucleic Acids Res., *26:* 3453–3459, 1998.
8. Rathinavelu, P., Malave, A., Raney, S. R., Hurst, J., Roberson, C. T., and Rathinavelu, A. Expression of mdm-2 oncoprotein in the primary and metastatic sites of mammary tumor (GI-101) implanted athymic nude mice. Cancer Biochem. Biophys., *17:* 133–146, 1999.
9. Shiina, H., Igawa, M., Shigeno, K., Yamasaki, Y., Urakami, S., Yoneda, T., Wada, Y., Honda, S., and Nagasaki, M. Clinical significance of mdm2 and p53 expression in bladder cancer. A comparison with cell proliferation and apoptosis. Oncology, *56:* 239–247, 1999.
10. Sato, N., Mizumoto, K., Maehara, N., Kusumoto, M., Nishio, S., Urashima, T., Ogawa, T., and Tanaka, M. Enhancement of drug-induced apoptosis by antisense oligodeoxynucleotides targeted against Mdm2 and p21WAF1/CIP1. Anticancer Res., *20:* 837–842, 2000.
11. Wang, H., Zeng, X., Oliver, P., Le, L. P., Chen, J., Chen, L., Zhou, W., Agrawal, S., and Zhang, R. *MDM2* oncogene as a target for cancer therapy: an antisense approach. Int. J. Oncol., *15:* 653–660, 1999.
12. Midgley, C. A., Desterro, J. M., Saville, M. K., Howard, S., Sparks, A., Hay, R. T., and Lane, D. P. An N-terminal p14ARF peptide blocks Mdm2-dependent ubiquitination *in vitro* and can activate p53 *in vivo*. Oncogene, *19:* 2312–2323, 2000.
13. Buzaid, A. C., Ross, M. I., and Soong, S. Classification and staging. *In:* C. M. Balch, A. N. Houghton, A. J. Sober, and S. Soong (eds.), Cutaneous Melanoma, Ed. 3, pp. 37–49. St. Louis, MO: Quality Medical Publishing, Inc., 1998.
14. Kononen, J., Bubendorf, L., Kallioniemi, A., Barlund, M., Schraml, P., Leighton, S., Torhorst, J., Mihatsch, M. J., Sauter, G., and Kallioniemi, O. P. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat. Med., *4:* 844–847, 1998.
15. Kobayashi, Y., Esato, K., Noshima, S., Oga, A., and Sasaki, K. Detection of 20q13 gain by dual-color FISH in breast cancers. Anticancer Res., *20:* 531–535, 2000.
16. Brodeur, G. M., and Hogarty, M. D. Gene amplification in human cancers: biological and clinical significance. *In:* B. Vogelstein and K. W. Kinzler (eds.), The Genetic Basis of Human Cancer, pp. 161–172. New York: McGraw-Hill, Inc., 1998.
17. Chen, J., Marechal, V., and Levine, A. J. Mapping of the p53 and mdm-2 interaction domains. Mol. Cell. Biol., *13:* 4107–4114, 1993.

18. Cordon-Cardo, C., Latres, E., Drobnjak, M., Oliva, M. R., Pollack, D., Woodruff, J. M., Marechal, V., Chen, J., Brennan, M. F., and Levine, A. J. Molecular abnormalities of *mdm2* and *p53* genes in adult soft tissue sarcomas. Cancer Res., *54:* 794–799, 1994.
19. Lianes, P., Orlow, I., Zhang, Z. F., Oliva, M. R., Sarkis, A. S., Reuter, V. E., and Cordon-Cardo, C. Altered patterns of MDM2 and TP53 expression in human bladder cancer. J. Natl. Cancer Inst., *86:* 1325–1330, 1994.
20. Osman, I., Scher, H. I., Zhang, Z. F., Pellicer, I., Hamza, R., Eissa, S., Khaled, H., and Cordon-Cardo, C. Alterations affecting the p53 control pathway in bilharzial-related bladder cancer. Clin. Cancer Res., *3:* 531–536, 1997.
21. Osman, I., Drobnjak, M., Fazzari, M., Ferrara, J., Scher, H. I., and Cordon-Cardo, C. Inactivation of the p53 pathway in prostate cancer: impact on tumor progression. Clin. Cancer Res., *5:* 2082–2088, 1999.
22. Osman, I., Sherman, E., Singh, B., Scher, H., Zelefsky, M., Shah, J., Cordon-Cardo, C., and Pfister, D. J. Inactivation of p53-pathway in squamous cell carcinoma of the head and neck: Impact on treatment outcome in patients treated with larynx preservation intent. Proc. Am. Soc. Clin. Oncol., *20:* Part 1 of 2, 228a (Abstract 910), 2001.
23. Oliner, J. D., Kinzler, K. W., Meltzer, P. S., George, D. L., and Vogelstein, B. Amplification of a gene encoding a p53-associated protein in human sarcomas. Nature (Lond.), *358:* 80–83, 1992.
24. Hoos, A., Uris, M. J., Stojadinovis, A., Mastorides, A., Dudas, M. E., Leung, D. H., Kuo, D., Brennan, M. F., Lewis, J. J., and Cordon-Cardo, C. C. Validation of tissue microarrays for immunohistochemical profiling of cancer specimens using the example of human fibroblastic tumors. Am. J. Pathol., *158:* 1245–1251, 2001.
25. Camp, R. L., Charette, L. A., and Rimm, D. L. Validation of tissue microarray technology in breast carcinoma. Lab. Investig., *80:* 1943–1949, 2000.
26. Bastian, B. C., LeBoit, P. E., Hamm, H., Brocker, E. B., and Pinkel, D. Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. Cancer Res., *58:* 2170–2175, 1998.
27. Bastian, B. C., Kashani-Sabet, M., Hamm, H., Godfrey, T., Moore, D. H., II, Brocker, E. B., LeBoit, P. E., and Pinkel, D. Gene amplifications characterize acral melanoma and permit the detection of occult tumor cells in the surrounding skin. Cancer Res., *60:* 1968–1973, 2000.
28. Volkenandt, M., Schlegel, U., Nanus, D. M., and Albino, A. P. Mutational analysis of the human *p53* gene in malignant melanoma. Pigment Cell Res., *4:* 35–40, 1991.
29. Polsky, D., Busam, K., Ren, Z., Dias, B., and Cordon-Cardo, C. Overexpression of MDM-2 is a frequent and early event in melanoma development. Proc. Annu. Meet. Am. Assoc. Cancer Res., *40:* 600, 1999.
30. Sauroja, I., Smeds, J., Vlaykova, T., Kumar, R., Talve, L., Hahka-Kemppinen, M., Punnonen, K., Jansen, C. T., Hemminki, K., and Pyrhonen, S. Analysis of G_1/S checkpoint regulators in metastatic melanoma. Genes Chromosomes Cancer, *28:* 404–414, 2000.
31. Hernberg, M., Turunen, J. P., von Boguslawsky, K., Muhonen, T., and Pyrhonen, S. Prognostic value of biomarkers in malignant melanoma. Melanoma Res., *8:* 283–291, 1998.
32. Gelsleichter, L., Gown, A. M., Zarbo, R. J., Wang, E., and Coltrera, M. D. p53 and mdm-2 expression in malignant melanoma: an immunocytochemical study of expression of p53, mdm-2, and markers of cell proliferation in primary *versus* metastatic tumors. Mod. Pathol., *8:* 530–535, 1995.
33. Poremba, C., Yandell, D. W., Metze, D., Kamanabrou, D., Bocker, W., and Dockhorn-Dworniczak, B. Immunohistochemical detection of p53 in melanomas with rare *p53* gene mutations is associated with mdm-2 overexpression. Oncol. Res., *7:* 331–339, 1995.
34. Gorgoulis, V. G., Zacharatos, P., Kotsinas, A., Mariatos, G., Liloglou, T., Vogiatzi, T., Foukas, P., Rassidakis, G., Garinis, G., Ioannides, T., Zoumpourlis, V., Bramis, J., Michail, P. O., Asimacopoulos, P. J., Field, J. K., and Kittas, C. Altered expression of the cell cycle regulatory molecules pRb, p53 and MDM2 exert a synergetic effect on tumor growth and chromosomal instability in non-small cell lung carcinomas (NSCLCs). Mol. Med., *6:* 208–237, 2000.
35. Li, X. J., Murai, M., Koyama, T., Wang, D. Y., and Hashimoto, K. MDM2 overexpression with alteration of the p53 protein and gene status in oral carcinogenesis. Jpn. J. Cancer Res., *91:* 492–498, 2000.
36. Abdel-Fattah, G., Yoffe, B., Krishnan, B., Khaoustov, V., and Itani, K. MDM2/p53 protein expression in the development of colorectal adenocarcinoma. J. Gastrointest. Surg., *4:* 109–114, 2000.
37. Capoulade, C., Bressac-de Paillerets, B., Lefrere, I., Ronsin, M., Feunteun, J., Tursz, T., and Wiels, J. Overexpression of MDM2, due to enhanced translation, results in inactivation of wild-type p53 in Burkitt's lymphoma cells. Oncogene, *16:* 1603–1610, 1998.
38. Bueso-Ramos, C. E., Manshouri, T., Haidar, M. A., Yang, Y., McCown, P., Ordonez, N., Glassman, A., Sneige, N., and Albitar, M. Abnormal expression of MDM-2 in breast carcinomas. Breast Cancer Res. Treat., *37:* 179–188, 1996.
39. Landers, J. E., Cassel, S. L., and George, D. L. Translational enhancement of *mdm2* oncogene expression in human tumor cells containing a stabilized wild-type p53 protein. Cancer Res., *57:* 3562–3568, 1997.
40. Shaulian, E., Resnitzky, D., Shifman, O., Blandino, G., Amsterdam, A., Yayon, A., and Oren, M. Induction of Mdm2 and enhancement of cell survival by bFGF. Oncogene, *15:* 2717–2725, 1997.
41. Ries, S., Biederer, C., Woods, D., Shifman, O., Shirasawa, S., Sasazuki, T., McMahon, M., Oren, M., and McCormick, F. Opposing effects of Ras on p53: transcriptional activation of mdm2 and induction of p19ARF. Cell, *103:* 321–330, 2000.
42. Halaban, R. Growth factors and melanomas. Semin. Oncol., *23:* 673–681, 1996.
43. Eton, O., Legha, S. S., Moon, T. E., Buzaid, A. C., Papadopoulos, N. E., Plager, C., Burgess, A. M., Bedikian, A. Y., Ring, S., Dong, Q., Glassman, A. B., Balch, C. M., and Benjamin, R. S. Prognostic factors for survival of patients treated systemically for disseminated melanoma. J. Clin. Oncol., *16:* 1103–1111, 1998.
44. Kakehi, Y., Ozdemir, E., Habuchi, T., Yamabe, H., Hashimura, T., Katsura, Y., and Yoshida, O. Absence of p53 overexpression and favorable response to cisplatin-based neoadjuvant chemotherapy in urothelial carcinomas. Jpn. J. Cancer Res., *89:* 214–220, 1998.
45. el-Deiry, W. S. Role of oncogenes in resistance and killing by cancer therapeutic agents. Curr. Opin. Oncol., *9:* 79–87, 1997.