Cyclin D1 Is a Candidate Oncogene in Cutaneous Melanoma

Edward R. Sauter, Un-Cheol Yeo, Andrea von Stemm, Weizhu Zhu, Samuel Litwin, David S. Tichansky, Giuseppa Pistritto, Mark Nesbit, Dan Pinkel, Meenhard Herlyn, and Boris C. Bastian


ABSTRACT

The retinoblastoma pathway has been implicated in melanoma; however, previous studies of one of the key components of this pathway, cyclin D1 (CD1), failed to find amplification of this gene in a large series of melanomas. We have recently shown that a particular subtype of melanoma, acral melanoma (AM), has frequent amplification of the CD1 locus. This suggested that CD1 might be important in AM and that it may also be important in other melanoma types, even though its copy number may not be altered. We compared CD1 gene copy number and protein expression in 137 invasive primary cutaneous melanomas (71 superficial spreading melanomas, 17 nodular melanomas, 19 lentigo maligna melanomas, 18 AMs, and 12 unclassifiable melanomas) using fluorescence in situ hybridization and immunohistochemistry. We found frequent amplification of CD1 in AM (44.4%) and occasional amplification in lentigo maligna melanoma (10.5%) and superficial spreading melanoma (5.6%). CD1 protein was overexpressed in all cases with amplifications and in an additional 20% of cases without amplification. We tested the importance of CD1 in cell growth in melanoma by using adenovirus-mediated antisense treatment targeted to CD1 in two melanoma cell lines, one with and the other without CD1 amplification and overexpression. Antisense mediated down-regulation of CD1 induced apoptosis in vitro and led to significant tumor shrinkage of melanoma xenografts in severe combined immunodeficient mice. However, it did not alter the growth of normal melanocytes. Together, these results suggest that CD1 may be an oncogene in melanoma and that targeting its expression may be therapeutically beneficial.

INTRODUCTION

Altered regulation of the G1-S transition of the cell cycle is common, if not essential, in the pathogenesis of cutaneous melanoma. Germ-line mutations that inactivate the p16/INK4a gene, a negative regulator of the G1-S checkpoint, or activate the positive regulator CDK4 are found in about 25% of melanoma families (1, 2). In sporadic melanoma, the INK4a region is deleted in 50–80% of cases (3–6), and mutations of CDK4 have been described (7). The p16-cyclin D/CDK4-Rb pathway is functionally altered in virtually all melanoma cell lines (8).

Additional important genetic lesions in melanoma affect the MAPK pathway (9). Ras mutations are found in about 25% of primary melanomas, and overexpression of receptor tyrosine kinases such as epidermal growth factor receptor and platelet-derived growth factor receptor α (10, 11) or receptor tyrosine kinase ligands such as platelet-derived growth factor, basic fibroblast growth factor, and HGF (12, 13) is also frequently found in melanoma.

MAPK and Rb pathway interconnect at the level of cyclin D. The CD1 promoter acts as a sensor for growth signals conveyed via the MAPK cascade and links this pathway to the cell cycle machinery (14). D-type cyclins positively regulate the activity of CDKs, leading to phosphorylation of Rb and promoting entry into mitosis (15).

The CD1 gene CCND1 is an important cancer gene. It is overexpressed in 50% of human mammary carcinomas, and mice that lack the CD1 gene are resistant to breast cancer development induced by activation of the MAPK pathway via the ras or neu oncogenes (16). Overexpression of CD1 is also found in other malignancies and, in general, can occur through several mechanisms. In parathyroid adenomas and mantle cell lymphomas, CD1 is activated by translocation. In many cancers including colon, breast, lung, head and neck, and bladder cancer, CD1 is activated by gene amplification (17–20).

In melanoma, the role of CD1 is less well established. In a series of 61 melanoma metastases, no CD1 amplifications were found (21). However, in a previous study of AM, we found frequent amplifications of chromosome 1q13 that encompassed the CD1 locus (6, 22). AM is a unique subtype of melanoma that occurs on the palms and soles, as well as under the nails, and does not seem to be related to UV irradiation. These studies suggested a possible role for CD1 in both AMs and non-AMs because one SSM and one LMM were found to have amplifications at 1q13 (6, 22). We also found amplifications of CD1 in a mouse model of melanoma using p53-deficient mice (23). In this study, not only did tumors with CD1 amplification overexpress the protein, but the majority of tumors with normal copy numbers also overexpressed CD1. Together, these findings motivated us to initiate a more thorough investigation of CD1 in human primary cutaneous melanoma.

MATERIALS AND METHODS

Cases and Tissue Arrays. Tissue microarrays were assembled from 139 invasive primary cutaneous melanomas as described previously (24). All cases were retrieved from the archives of the Dermatopathology Section of the University of California, San Francisco and the Department of Dermatology, University of Würzburg. Ninety-one of these were retrieved randomly according to the following criteria: all were primary melanomas with a tumor thickness of ≥1 mm. The other cases were primary cutaneous melanomas that had previously been studied by CGH (6, 22). In addition, we used 19 melanocytic nevi (6 congenital, 6 dysplastic, and 7 common types) as controls for FISH and immunohistochemistry.

FISH Analysis of Formalin-fixed Tissue Microarray Sections. Dual-color FISH was carried out on sections of the tissue array as described previously (25). Genomic clones containing the genes for CD1 (clone 204A) and FGF4/FGF3 (clone 333A) were provided by Vysis Inc. (Downers Grove, IL). A reference probe for chromosome 11p (clone RMC11B022) was obtained from the University of California, San Francisco laboratory’s resource. Probes were labeled with Cy3 (Amersham, Arlington Heights, IL) or digoxigenin (Boehringer Mannheim, Indianapolis, IN) by nick-translation. FISH signals were scored with a fluorescence microscope (Zeiss Jena, Germany) using a ×63 objective. Criteria for amplification was ≥2.5-fold more test probe signals than reference signals in at least 30% of the tumor cells.
**Immunohistochemistry.** Expression of CD1 was determined using the mAb ASM29 from Zymed (dilution, 1:200; South San Francisco, CA) according to standard procedures using 3-amin-9-ethylcarbazole as a chromagen. Immunoreactivity was assessed using the >20 objective, and only cells that showed definitive nuclear staining were counted. Scores were recorded semiquantitatively as follows: (a) 1+, 10–25% of nuclei stained; (b) 2+, 25–50% of nuclei stained; (c) 3+, 50–75% of nuclei stained; and (d) 4+, >75% of nuclei stained. For statistical analysis, scores over 1+ were regarded as positive.

**Data Analysis and Statistics.** CGH interpretation, FISH spot counting, and immunohistochemistry scoring were performed blinded, and data were compiled separately. Analyses were computerized. Contingency table analysis was used to test for association of the parameters. Fisher’s exact test was used if any of the expected values were <5. P < 0.05 was regarded as statistically significant.

**Cell Culture.** Melanocytes were cultured in MCDIB153 (Sigma Chemical Co., St. Louis, MO) supplemented with 2% fetal bovine serum, 10% chelated FCS, 2 mM glutamine (Mediatech, Herndon, VA), 20 μM choleratoxin (Sigma Chemical Co., St. Louis, MO) supplemented with 2% fetal bovine serum, 10% chelated FCS, 2 mM glutamine (Mediatech, Herndon, VA), and grown in DMEM supplemented with 10% FCS. All tissue culture reagents were purchased from Sigma Chemical Co.

**Construction of Replication-defective Adenoviral Vectors.** The construction of AS CD1 has been described previously in detail (31). Briefly, 20-mer primers designed to amplify CD1 were PCR-amplified for 35 cycles. The 1.1-kb product including the entire CD1 open reading frame was inserted into the shuttle vector pAd.CMV-LINK.1 (University of Pennsylvania Vector Core). pAd.CMV-LINK1 was then cotransfected into 293 cells with adenoviral DNA lacking the E1 and E3 regions (32) using calcium phosphate precipitation. Homologous recombination between pAd.CMV-LINK1 and d7001, resulting in the incorporation of CD1 into the adenoviral backbone, led to a cytopathic effect as evidenced by plaque formation after approximately 2 weeks. The virus was purified by cesium chloride centrifugation and dialyzed against PBS before use. d7001 is an adenovirus type 5 with a large E3 deletion, allowing the insertion of genes of interest. It has been shown to replicate to the same titers as native adenovirus type 5 in cultured cells (32).

**Protein Isolation and Analysis of CD1 Expression.** Protein isolation and Western blotting were performed both to evaluate baseline expression of 451 Lu and 1205 Lu compared with normal melanocytes and to determine whether AS CD1 down-regulated CD1 protein expression. Total protein was calculated and equalized for each loaded lane of the two gels (one gel for baseline expression, and a second gel to determine the effect of AS CD1). To evaluate baseline expression, cells were serum-starved for 24 h before protein isolation to minimize the effects of serum on CD1 expression. To determine the effect of AS CD1 on CD1 expression, 1205 Lu cells were either not treated (mock-treated) or treated with 50 pfu/cell LacZ (control virus) or AS CD1. Forty-eight h after viral transduction, a SDS-based single lysis buffer was added to the cells, the resulting solution was centrifuged, and the supernatant was decanted. Total protein concentration of the supernatant was determined using the Pierce BCA Protein Assay Reagent Kit (Rockford, IL). Samples (65 μg) were separated electrophoretically under reducing conditions in a discontinuous 12% polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. This procedure was performed twice. After transfer, membranes were blocked with 5% nonfat dry milk. The first membrane was probed with a mouse mAb to β-actin (1:5000; clone AC-15; Sigma Chemical Co.), and the second membrane was probed with a mouse mAb to CD1 (1:200; clone HD-11; Santa Cruz Biotechnology, Santa Cruz, CA) followed by a phosphatase-conjugated goat antimouse IgG (1:1000; Jackson ImmunoResearch, West Grove, PA). After washing, the substrates 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium were added, and the membrane was observed until a signal was detected.

**In Vitro Growth.** The effect of AS CD1 on proliferation was evaluated by measuring the incorporation of 1H]thymidine. Two melanoma cell lines (451 Lu and 1205 Lu) and normal melanocytes were evaluated. Cells were seeded at a concentration of 2 x 10^4 cells in 200 μL melanoma growth media/well. Each treatment at each viral titer was evaluated in four wells. Twelve to 24 h after seeding, the cells were mock-infected or infected with LacZ or AS CD1. To identify an optimal viral dose for each of the cell lines, four different titers (1, 10, 50, and 100 pfu/cell) of each virus were tested for each cell line. Thirty h after viral administration, 1 μCi of [1H]thymidine was added per well, and an 18-h incubation period was carried out. After the media were removed, the cells were detached with 0.06% trypsin/EDTA and harvested with a Filtermate 196 Cell Harvester (Packard Instrument Company, Meriden, CT), and a Matrix 9600 Direct Beta Counter (Packard) was used to measure the number of counts/well/basal min. Wilcoxon’s rank-sum test was used to determine whether there was a significant difference in growth between the groups (mock-, LacZ-, or AS CD1-treated).

**In Vivo Growth.** 451 Lu and 1205 Lu cells were grown in melanoma media to 70–80% confluence, detached with 0.06% trypsin/EDTA, and counted, and 10^5 cells were injected s.c. into the dorsal hindquarter of severe combined immunodeficient mice just medial to the right hind limb. After tumors had grown to a minimum volume of 75 mm^3, one or two viral constructs (each consisting of 5 x 10^6 pfu virus in 50 μl of melanoma media) were injected within each tumor at baseline using a tuberculin syringe. A second dose of one viral construct was administered 7 days after baseline to a subset of the tumors. Each treatment was administered to four to eight mice. The treatments administered were LacZ, AS CD1, LacZ + LacZ, LacZ + AS CD1, and AS CD1 + AS CD1. Each tumor was measured five times (at baseline and 4, 7, 10, and 14 days after infection). Some tumors were observed for longer periods if the tumor burden was acceptably small. All mice were sacrificed by day 31. Tumor volumes at these five times were fitted to the curve y = A exp(β t). The tumor regression rate β was thus estimated for each mouse from the five observations. The β values in each group of mice were rank ordered and submitted to the Wilcoxon two-sample (one-sided) procedure to test the hypothesis of equal rates of tumor regression among groups versus the alternative hypothesis that treated tumors regress faster.

**Apoptosis.** For apoptosis, 451 Lu and 1205 Lu cells were grown to 70–80% confluence in melanoma media with 1% FCS. 1205 Lu cells were grown without serum (451 Lu cells did not grow well in serum-free media). For cells grown with serum, culture media was removed, and serum-free media were added before viral transduction. The cells were then mock-transduced or transduced with LacZ or AS CD1. A viral titer of 50 pfu/vector was used; thus, cells undergoing combined therapy received 100 pfu/cell. Cultured cells were detached 1, 2, 3, 4, and 5 days after transduction with 0.06% trypsin/EDTA and fixed in 70% ethanol. After pelleting and ethanol removal, the cells were resuspended in propidium iodide and left in the dark for a minimum of 20 min. Cell cycle analysis, including the sub-G0, or apoptotic cell fraction, was then performed using an EPICS XL flow cytometer (Coulter Corp., Hialeah, FL). Linear regression was applied to the 5-day data plots of each trial. A generalized likelihood ratio statistic was then used to compare the rates of cell apoptosis for treatment pairs. This generated a χ^2 statistic with 1 degree of freedom. We then applied a Bonferroni adjustment for multiple comparisons to determine significance.

**TUNEL Staining.** To detect cells undergoing apoptosis, the peroxidase in situ cell death detection kit (Roche Molecular Biochemicals) was used. Briefly, tissue sections were deparaffinized by heating at 60°C for 30 min and washing twice in xylene for a total of 10 min. The sections were then rehydrated through a graded series of alcohols, treated with proteinase K (20 μg/ml) in 10 mM Tris-HCl (pH 8.0) for 30 min at room temperature, and washed with ice-cold PBS four times. Endogenous peroxidases were inactivated by incubating the sections in 3% H2O2 in methanol for 10 min at room temperature and washed again. Then, the labeling solution containing the enzyme terminal deoxynucleotidyl transferase (POD kit) was diluted 1:10 and added to the sections for 1 h at 37°C in a humidified chamber. For a negative control, the...
enzyme was omitted from the labeling solution. Then, samples were analyzed under a fluorescence microscope.

RESULTS

**Amplification of CD1.** FISH counts were obtained from 137 of 139 cases (98%). A total of 15 amplifications were found (11%). The average copy number was 7.2 (range, 4.5–15), and there was no significant difference in copy number between the two probes for 11q13. The average amplification level, as determined by the ratio of the copy number at 11q13 and the copy number at the reference locus on chromosome 11p15, was 3.9 (range, 2.5–7). None of the control cases showed copy number changes.

As shown in Table 1, there was a significant difference in the CD1 amplification frequency among different melanoma subtypes. Consistent with our previous report (22), the locus was frequently amplified in AM (44.4%), whereas other melanoma types had a lower frequency of amplifications. There was no significant difference in tumor thickness of cases with and without amplifications.

Because only a relatively small fraction of a tumor is represented in the tissue array, substantial heterogeneity of copy number in the tumor FISH could potentially lead to an underestimate of amplifications that were present in tumor areas not represented in the array. To address this issue, we compared the concordance of the copy number status at chromosome 11q13 as determined by FISH with previously acquired CGH data available from 52 cases. FISH and CGH results were highly concordant ($P < 0.001$). Thirty-nine cases (75%) had no detectable amplification by either method, 10 cases (19%) showed amplification by both CGH and FISH, and no cases showed amplification by FISH that did not also show an 11q13 amplification by CGH. Only in three cases (6%) did FISH not detect amplifications found by CGH. The majority (8 of 13) of cases that showed 11q13 amplifications also had large deletions of chromosome 9p that spanned the INK4a/ARF locus by CGH.

**Expression of CD1 Protein in Primary Melanomas and Mela-
noma Cell Lines.** Data for both CD1 gene copy number and CD1 expression levels were obtained from 108 cases. Heterogeneous expression within tumors possibly biasing the tissue array analysis was addressed by comparing CD1 expression levels in the array with corresponding whole sections of 28 randomly selected cases. The results were highly concordant ($P < 0.001$). Normal melanocytes in histologically normal skin adjacent to the tumors did not show any CD1 expression consistent with previous reports (33, 34). Of the 19 melanocytic nevi that were used as controls only 1 case, a dysplastic nevus, expressed CD1 (1+).

All cases with CD1 amplification showed CD1 protein expression by immunohistochemistry (Table 1). In addition, 19 (17.6%) of the cases with normal CD1 copy number overexpressed CD1 protein, indicating that additional factors other than copy number levels influenced expression. The finding that 4 of the 28 cases for which whole sections were studied for CD1 expression had heterogeneous CD1 expression throughout the specimen supported this notion. Two of the cases with heterogeneous expression showed CD1 amplification. In these two cases, the copy number levels in the tumor only partially paralleled the expression pattern. Fig. 1 illustrates one of these cases, showing a strikingly polar expression pattern in which the immunoreactivity decreased from the superficial to the deeper parts of the tumor (Fig. 1A). The tumor showed a sharply demarcated area with high copy numbers to the left (15.1 at the top to 15.4 at the bottom) and moderate copy numbers in the rest of the lesion (5.9–7.1). The intensity of the immunostain parallels the differences in copy number only within the horizontal plane. The striking vertical gradient of decreasing intensity is independent of the gene dosage and indicates the presence of tissue factors that affect expression levels.

CD1 expression was evaluated in melanocytes, 451 Lu cells, and 1205 Lu cells. Expression was similar in melanocytes and 1205 Lu cells, whereas it was somewhat higher (50% higher by densitometric analysis) in 451 Lu cells (Fig. 2A).

**CD1 and Proliferation Rate.** We used the Ki67 labeling index to assess the proliferation rate of the melanomas studied. Data pairs of CD1 gene copy number and Ki67 labeling were available for 114 cases. There was a trend for cases with CD1 amplification to have higher proliferation rates (Fisher’s exact test, $P = 0.085$). There was a slightly stronger association between CD1 expression levels and Ki67 labeling rate (107 data pairs; Fisher’s exact test, $P < 0.05$; cutoff levels, >1 for CD1 and Ki67 expression), presumably due to the cases in which CD1 was overexpressed but not amplified. We also compared the labeling indices of tissue array biopsies and corresponding whole sections. The 19 cases studied yielded similar results (Fisher’s exact test, $P < 0.05$).

**Effects of Inhibition of CD1 Expression on Tumor Growth.** To study the relevance of CD1 for melanoma tumor growth, we selected two tumorigenic cell lines. 451 Lu cells were amplified at 11q13 by CGH, and the amplification level as determined by FISH was 2.5. 1205 Lu cells had a normal copy number at the CD1 locus by both CGH and FISH.

CD1 protein expression in 1205 Lu cells decreased by 78% 48 h after transduction with AS CD1 as determined by densitometric analysis of a Western blot (Fig. 2B), compared with 1205 Lu cells 48 h after transduction with the control vector LacZ or mock-treated cells. The decreased expression of the mature CD1 protein after
treatment with AS CD1 was paralleled by the detection of a 21-kDa band recognized by the CD1 mAb (Fig. 2B). A similar band was observed after treating SCCs with AS CD1 (32). To determine whether the 21-kDa band was more likely a functional truncated protein or a breakdown product, we again mock-infected or infected SCC cells with LacZ or AS CD1 and waited 48 h before harvest. With overnight exposure to substrate, a 21-kDa band was observed in each of the three lanes. From this, we surmised that the smaller band detected in both control and treated cells was more likely a breakdown product of CD1 than a functional truncated protein.

The in vitro dose-response growth profile assessed by [3H]thymidine uptake after treatment of normal melanocytes, 451 Lu, and 1205 Lu is indicated in Fig. 3. An optimal viral titer was determined for both tumor cell lines, defined as the maximal effect of AS CD1 in decreasing cell growth with minimal effect of LacZ on cell growth compared with mock-infected cells. The optimal titer was 50 pfu/cell for 1205 Lu and 100 pfu/cell for 451 Lu. At the optimal viral titer, AS CD1 led to a 97% reduction in growth compared with LacZ for 451 Lu and 1205 Lu (P = 0.014 for each). AS CD1 did not inhibit the growth of normal melanocytes.

To assess the effect of CD1 inhibition on tumor growth in vivo, we studied the effect of AS CD1 treatment on tumors grown from 451 Lu or 1205 Lu cells in nude mice. Treatment of both 451 Lu (P = 0.0048) and 1205 Lu (P = 0.004) tumors with AS CD1 led to significant growth suppression as compared with control treatment with the LacZ-expressing (control) vector (Fig. 4). We also sought to determine whether doubling the dose of AS CD1 led to a greater response. Doubling the viral dose for LacZ did not alter tumor growth compared with a single dose of LacZ. AS CD1 + AS CD1 suppressed 1205 Lu tumor growth compared with LacZ + LacZ (P = 0.0022), but not compared with AS CD1 alone (P = 0.4). We also evaluated the effect of repeat dosing of LacZ or AS CD1 on tumor growth. Compared with the growth observed after a single treatment with LacZ or AS CD1, there was no significant effect of a second dose of virus administered 7 days after the first injection.

Transduction with AS CD1 Induces Apoptosis. Many studies have suggested that serum suppresses adenoviral entry into cells. As a result, we evaluated the effect of treatment in the presence and absence of serum for 1205 Lu cells. In the absence of serum, AS CD1 significantly increased the rate of apoptosis (P < 0.0001) in 1205 Lu cells when compared with LacZ and mock infection. In the presence of 1% FCS, 1205 Lu cells underwent apoptosis more slowly (P < 0.05) compared with cells grown without serum but underwent apoptosis more rapidly (P < 0.0001) than LacZ control. 451 Lu cells grew poorly without serum; therefore, they were evaluated only when grown in 1% FCS. In the presence of 1% FCS, they showed a significant increase in apoptosis after treatment with AS CD1 when compared with LacZ treatment (P < 0.0001).
To identify cells that underwent apoptosis in vivo, we used TUNEL staining. Treatment with AS CD1 resulted in a highly increased number of TUNEL-positive cells in both 451 Lu and 1205 Lu tumors (Fig. 5C). By contrast, few TUNEL-positive cells were present in both 451 Lu and 1205 Lu cells treated with a LacZ control. TUNEL-positive cells were always surrounded by unstained cells, and there was no indication of aggregation of apoptotic cells into clusters.

**DISCUSSION**

In this study, we have shown that CD1 is recurrently amplified and overexpressed in primary melanomas and that down-regulation of its expression has a significant impact on melanoma cell growth. AMs had significantly higher amplification frequencies compared with other melanoma types, which is consistent with the overall high rate of amplification in AMs (22). The relatively low frequency of amplification in nonacral types of melanoma, representing the vast majority of melanoma in Caucasians, may explain why a previous study of 61 sporadic metastatic melanomas and 5 melanoma cell lines did not detect any CD1 amplifications (21). We found that, independent of the type of melanoma, all cases with CD1 amplification also overexpressed CD1 protein. A similar pattern was seen in a mouse model of melanoma, where we also observed copy number increases and overexpression of CD1 (23). Similar to the mouse model, we also found overexpression in about 20% of cases with normal CD1 copy number (20% of LMMs and 17% of SSMs). This indicates that, as found for other cancer genes, expression levels can be modulated by mechanisms different from gene dosage. One example of an epigenetic mechanism that regulates CD1 expression is mitogen-induced Ras signaling, which promotes transcription of the CD1 gene via a kinase cascade that depends on the sequential activities of Raf-1, MAPK kinase 1 and 2, and extracellular signal-regulated protein kinases (15).

The fact that all cases with amplification overexpressed CD1 protein indicates that CD1 is a target gene of the amplification of 11q13. However, because other candidate oncogenes map to this region, we cannot rule out the possibility that other genes may contribute to the selection forces driving the amplification. To evaluate CD1 as a candidate oncogene, we studied the effect of AS-mediated down-regulation of CD1 expression in two melanoma cell lines. We found that AS CD1 reduced growth and induced apoptosis in vitro and led to significant shrinkage of melanoma xenografts, similar to previous reports in which we demonstrated that AS CD1 also induced apoptosis in squamous carcinoma cells, including five cell lines that contained an amplification of CD1 and overexpressed the protein (35). The dramatic in vitro effect of AS CD1 is...
somewhat surprising because CD1 is dispensable for the proliferation of most cells, as shown by studies of CD1-null mice (36). Also, normal melanocytes were not affected by AS CD1, indicating that in melanocytes, CD1 is not essential as in e.g., mammary gland epithelial cells (36). The effect of AS CD1 on melanoma cells might indicate that the tumors have become dependent on CD1 expression and that its reduction is incompatible with cell survival. The effect on tumors, although dramatic, was perhaps less than would have been predicted from our in vitro results. This could be explained by the limitations of uniform administration of agent in vivo due to intervening stroma and diffusion of virus through tissue versus in culture media.

Other reports also indicate that CD1 may not only be a tumor cell growth promoter but may also function as a survival factor for tumor cells and that CD1 down-regulation may result in apoptosis (37). CDK4-CD1 complexes serve as a sink for p21\(^{Cip1}\) and p27\(^{Kip1}\) proteins (15). Reducing cellular CD1 levels thus would lead to a release of p21\(^{Cip1}\) and p27\(^{Kip1}\) proteins. Increased p21\(^{Cip1}\) levels after AS CD1 treatment have been observed (37), and we noted increased p21\(^{Cip1}\) expression levels in the xenograft tumors after AS CD1 treatment in our study (data not shown). Besides inhibiting CKK2-cyclin E complexes, p21\(^{Cip1}\) can have proapoptotic properties (38) and thus could represent one mechanism of how AS CD1 treatment induces apoptosis. The effect of CD1 withdrawal may be dependent on functional Rb because in a previous study we found that a cervical SCC cell line that lacks functional Rb was resistant to AS CD1-mediated growth suppression (31).

It is interesting to note that the majority of cases that showed 11q13 amplification also had large deletions of chromosome 9p by CGH, which spanned the CDKN2A locus at chromosome 9p21 (encoding INK4a/ARF). If one assumes that p16 is a target of these deletions, one might expect that CD1 amplification and deletion of 9p21 were mutually exclusive because their effects are commonly viewed as being redundant. Another recent study on SCCs of the larynx also found frequent association of p16 mutation/deletion/methylation and
CD1 amplification/overexpression, which contradicts this view (39). However, it is also conceivable that the target of the 9p21 deletions in CD1-overexpressing tumors is ARF because previous studies in p53-deficient mice have shown a link between the ARF/P53 pathway and CD1 overexpression (23). Future studies are warranted to study this pathway in melanomas with CD1 overexpression and especially AMs.

In summary, our data strongly implicate CD1 as an oncogene in melanoma, making it a candidate for further evaluation as a therapeutic target in this disease.

ACKNOWLEDGMENTS

We thank Susan Charzan for excellent technical assistance.

REFERENCES


Cyclin D1 Is a Candidate Oncogene in Cutaneous Melanoma

Edward R. Sauter, Un-Cheol Yeo, Andrea von Stemm, et al.


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/11/3200

Cited articles  This article cites 38 articles, 18 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/11/3200.full#ref-list-1

Citing articles  This article has been cited by 24 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/62/11/3200.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.