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Research Paper

Increased Gene Copy Number of The Transcription Factor E2F1 in Malignant Melanoma

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E2F1, 20q11, melanoma, fish, gene amplification

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NOTE

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

ABSTRACT

Translocations and unique chromosome break points in melanoma will aid in the identification of the genes that are important in the neoplastic process. We have previously shown a unique translocation in malignant melanoma cells der(12)t(12;20). The transcription factor E2F1 maps to 20q11. Increased expression of E2F has been associated with the autonomous growth of melanoma cells, however, the molecular basis has not yet been elucidated. To this end, we investigated E2F1 gene copy number and structure in human melanoma cell lines and metastatic melanoma cases. Fluorescent in situ hybridization (FISH) analysis using a specific E2F1 probe indicated increased E2F1 gene copies in melanoma cell lines compared to normal melanocytes. We also observed increased copies of the E2F1 gene in lymph node metastases of melanoma. In addition, Western blot analysis demonstrated increased E2F1 protein levels in 8 out of 9 melanoma cell lines relative to normal melanocytes. Inhibition of E2F1 expression with RNAi also reduced melanoma cell growth. Our results suggest that the release of E2F activity by elevated E2F1 gene copy numbers may play a functional role in melanoma growth.

INTRODUCTION

Malignant melanoma will affect more than 50,000 people in the United States this year and result in over 7,000 deaths.[1] The incidence of malignant melanoma is among the most rapidly rising of all malignancies.[2] If diagnosed early, melanoma is highly curable by surgical excision. However, in individuals with deep local invasion, or with spread to lymph node or distant sites, the disease is highly resistant to all forms of therapy. The median survival for patients with metastatic melanoma is 6–9 months.[3]

Utilizing genomic technology in melanoma research will aid in the identification of novel disease related genes. Previous studies by our group using spectral karyotyping (SKY) and FISH indicate that malignant melanoma had increased copies of the long arm of chromosome 20.[4] Furthermore, we have been able to demonstrate a unique translocation der(12)t(12;20)(q13;q11) in melanoma cells. These studies suggest that 20q11 may contain one or more putative oncogenes which may play important roles in the development or the progression of malignant melanoma.

Altered regulation 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 G_1-S checkpoint, or activate CDK4, a positive regulator, are found in about 25% of melanoma families.[5] In sporadic melanoma, the *INK4a* locus is deleted in 50–80% of cases[6] and mutations of CDK4 have been described.[7] Cell cycle progression is regulated by the accumulation and destruction of specific proteins. The E2F1 protein is a powerful transcription factor that mediates the transition from G_1 into S. The phosphorylation of the retinoblastoma protein (Rb) by CDK4 releases E2F1 from the inhibitory effects of Rb. Released E2F1 drives quiescent cells into S-phase. The phosphorylation of Rb and subsequent release of E2F1 is prevented by *p16-cyclin/INK4a*. The p16-cyclin D/CDK4-Rb pathway is functionally altered in virtually all melanoma cell lines.[8] However, exactly which components of this pathway are consistently altered remains to be clarified. Interestingly, alterations in E2F transcriptional activity have been associated with the autonomous growth of melanoma cells.[9]

On the basis that E2F1 maps to 20q11, a region we have found to be frequently altered in melanoma, and regulates the expression of numerous genes, the present study investigated the molecular basis for E2F1 deregulation in malignant melanoma. We report that the E2F1 gene is amplified in melanoma cell lines and archival metastatic lesions. We also found increased expression of E2F1 at the protein level. Finally, melanoma cells transfected

with an RNAi construct displayed decreased cell growth. These data for the first time show gene amplification of E2F1 in malignant cells and suggest a possible role for this protein in the aberrant growth of melanocytic malignancies.

MATERIALS AND METHODS

Cell lines and tumors. Normal melanocytes were purchased from Cascade Biologics (Portland, OR) and cultured according to the manufacturer's instructions. Normal dermal fibroblasts and melanoma cells were obtained from the Arizona Cancer Tissue Core Service (Tucson, AZ). The cell lines were cultured and maintained as previously described.[4] Formalin fixed paraffin embedded metastatic melanoma and invasive primary melanoma lesions were obtained from the University of Arizona and the University of Pittsburgh Departments of Pathology. All aspects of these studies were approved by the University of Arizona (Tucson, AZ), University of Pittsburgh (Pittsburgh PA) and the National Institute of Occupational Safety and Health human institutional review boards (IRB).

Fluorescence in situ hybridization. Metaphase spreads were prepared from normal human lymphocytes and the malignant melanoma cell lines. When the cell lines were 70% confluent, 0.1 mg/ml to 100 mg/ml colcemid (Gibco/BRL, Rockville, MD) was added to block the cells in metaphase. Cell cultures that were resistant to colcemid were given the higher dose of colcemid. The lymphocyte cultures were blocked with 0.01 mg/ml colcemid after 72 hours of culture. The cell lines and lymphocytes were harvested and the slides were prepared as previously described.[4]

The whole chromosome Spectrum Red labeled painting probe for chromosome 20 (Vysis, Downers Grove, IL) was hybridized to the tumor cell line spreads to locate portions of the chromosome within the metaphase as described previously.[4] The P1 clone RMC PO88 was a generous gift from Dr. Joe Gray, Lawrence Livermore Laboratories. The P1 clone was labeled with Spectrum Green dUTP (Vysis, Downers Grove, IL) using the Vysis Nick Translation kit (Vysis, Downers Grove, IL) and hybridized following the manufacturer's directions (Vysis Downers Grove, IL) with modifications. The probe was mixed in LSI/WCP hybridization buffer (Vysis, Downers Grove, IL), COT 1 DNA (Sigma-Aldrich, St. Louis, MO) and placed at -20°C overnight. The location of the labeled probe was verified on metaphase spreads isolated from normal human lymphocytes. The slides and the probe were denatured at 80°C for two minutes, hybridized overnight at 43°C and washed for three times each for 5 minutes in 50.0% formamide/2XSSC at 45°C, then 0.1X SSC at 60°C followed by washes in 4X SSC/0.1% NP-40 at 45°C. Two independent observers (Sargent LM, Senft JR) analyzed at least 50 metaphase spreads for each sample. Formalin fixed tissue slides were processed following the Vysis protocol for paraffin embedded tissue slides with a few modifications. The slides were heated at 56°C for 4 hours to melt the paraffin, rinsed with distilled water to remove the excess paraffin and then treated with 1X Skipdewax solution (Insitus Biotechnologies, Albuquerque, NM) to remove the remaining paraffin. The slide was then placed in 0.02% protease in NaCl pH 2.0 at 37°C for 50 minutes. The slide and probe were denatured at 80°C for 15 minutes, hybridized at 37°C for 24 hours then washed and stained with DAPI using the manufacturer's standard protocol (Vysis Inc, Downers Grove, IL). Two independent observers (Sargent LM, Senft JR) analyzed at least 100 nuclei for every sample. Stromal cells were counted as a normal control for the hybridization.

Southern blot analysis. Genomic DNAs were digested with *BglII* and quantitated by a fluorimetric assay using 4, 6-diamidino-2-phenylindole to ensure equal loading in all lanes. Electrophoresis was performed in 0.8% agarose gels, and the DNA samples were transferred to nylon membranes by capillary blotting. The 1.0-kb SacII/Xho I fragment of the human E2F1 cDNA was radiolabled by random priming with [^{32}P]CTP (NEN/Dupont, Wilmington, DE). Hybridization was performed overnight at 42°C in buffer containing 5X SSC [1x SSC is 0.15 M NaCl, 0.015 M Sodium Citrate (pH 7.00, 50% formamide, 1.0% sodium dodecyl sulfate (SDS), 4x Denhardt's reagent, salmon sperm DNA (940 µg/ml), and 0.01 M Tris-HCl (pH 7.5)]. The membranes were washed twice in 0.1X SSC and 0.1% SDS at 42°C for 20 minutes and autoradiography performed for 48 or 96 hours at -70°C.

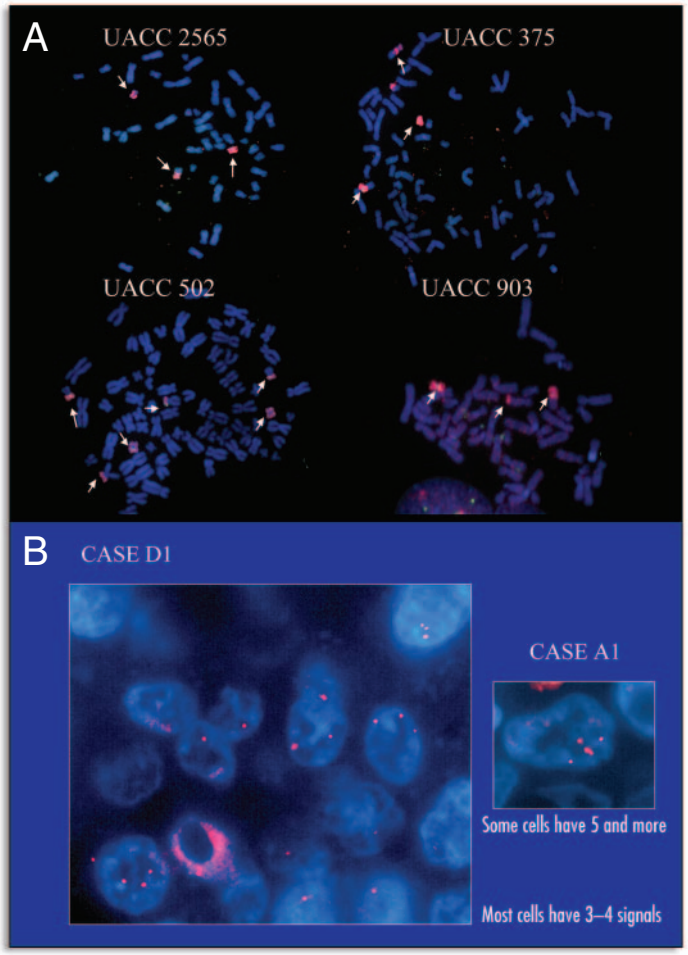

Figure 1. Flourescent in situ hybridization analysis of E2F1 in melanoma. (A) FISH analysis of metaphase spreads of melanoma cell lines. The P1 clone RMC P088 was labeled and hybridized as described in the methods to metaphase spreads isolated from the malignant melanoma tumor cell lines. Spectrum red dUTP whole chromosome painting probe for chromosome 20 (Vysis Inc, Downers Grove IL) was used to locate the chromosome within the spread. At least 50 metaphase spreads were analyzed for each tumor cell line. (B) Interphase FISH analysis of E2F1 in lymph node metastasis of malignant melanoma patient samples. The tissue was prepared and hybridized with the Spectrum Green dUTP labeled P1 clone RMC P088.

Western blot analysis. Protein extraction and Western blot analysis were performed as previously described.[10] Briefly, cell lysates were prepared by sonicating the cells for 30 seconds in RIPA buffer [10 mM Tris-HCL (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% v/v Nonidet P-40, 1% sodium deoxycholate, 1 mM sodium orthovandate, 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO). Lysates were cleared by centrifugation at 13,000 x g for 30 min at 4°C. Equal amounts of cell extracts (50 µg) were resolved on SDS-polyacrylamide gels and then transferred onto polyvinylidene difluoride membranes (BioRad Laboratories, Hercules, CA). After blocking membranes in 5% non-fat dry milk, they were incubated overnight with a 1/200 dilution of the KH20 and KH95 monoclonal primary antibodies (Upstate Biotechnology, NY). Membranes were then washed three times in 1XPBS containing 0.5% Tween 20 (PBS-T) for 20 minutes before incubation with the secondary horseradish peroxidase-linked antibody diluted in 1% bovine serum albumin PBST for 1 hour. Detection was performed using an enhanced chemiluminescence detection system (Amersham Biosciences, Piscataway, NJ).

Table 1 ***E2F1* gene copy number in melanoma cell lines**

Cell Line	E2F1 Copies # (FISH)	Expected # (G-band)
A 375	4 ± 0.3	3
UACC 375	6 ± 1.0	4
UACC 457	5 ± 0.7	4
UACC 502	6 ± 0.5	4
UACC 612	7 ± 1.0	3
UACC 647	6 ± 1.0	4
UACC 827	4 ± 0.1	3
UACC 903	3 ± 0.5	2
UACC 1022	9 ± 2.0	3
UACC 1065	4 ± 1.0	2
UACC 1460	4 ± 1.0	2
UACC 2565	3 ± 0.5	2

The copy number of E2F1 was determined by Fluorescent in situ hybridization. The probe RMCP088 was labeled with Spectrum Green dUTP and then hybridized to metaphase spreads as described in the methods. The average number of copies was determined. The expected number of copies of E2F1 was based on the modal karyotype as published in Sargent et al., 2001.[4]

Table 2 ***E2F1* gene copy number in metastatic malignant melanoma patient samples**

Sample #	E2F1 Copies # (FISH)	Expected # (Stroma)	Stain for E2F
1	3.3 ± 0.5	2 ± 0.2	4
2	4.0 + 0.6	2 + 0.5	4
3	3.0 + 1.0	2 + 0.2	3
4	3.0 + 0.5	2 + 0.6	4
5	4.0 + 1.0	2 + 0.5	4
6	2.2 + 0.6	2 + 0.5	1
7	4.0 + 0.6	2 + 0.6	4
8	3.0 + 0.4	2 + 0.2	4
8	4.0 + 0.5	2 + 0.3	*
10	3.2 + 0.4	2 + 0.1	3
11	3.3 + 0.1	2 + 0.2	3
12	3.5 + 0.3	2 + 0.3	3

*Insufficient amount of sample for analysis. Fluorescent in situ hybridization analysis of the copy number of E2F1 was performed on archival formalin fixed tissue slides as described in the methods. At least 100 cells were counted by two observers. Stromal cells were counted as a control. The average number of copies of E2F1 per cell was determined. The data was analyzed by Chi-square analysis.

Immunohistochemistry. Five micron sections prepared from formalin fixed paraffin embedded malignant melanoma patient samples were examined for expression of E2F1 by immunohistochemistry. Formalin fixed paraffin embedded one day old mouse samples were prepared by the same methods as a positive control for the expression of E2F1. The slides were processed to remove paraffin, rehydrated in xylene, dehydrated in ethanol, and the hidden antigen retrieved as previously described.[11] The slides were treated with 30.0% hydrogen peroxide, blocked with 5.0% goat serum for 30 minutes and then incubated at 4°C for 12 hours with a 1/1000 dilution of rabbit polyclonal IgG E2F1 antibody H137 (Santa Cruz, Santa Cruz, CA) and detected with an AEC staining kit (Dako, Carpenteria, CA) following manufacturer's protocol. Negative controls were incubated with the secondary antibody only. Lymphocytes and stromal cells were also analyzed as controls for the staining. Immunohistochemical scoring was evaluated as previously described by our group using methods described by Khan et al.[10,12] Briefly, the immunohistochemical score is as follows: No staining is scored as 0; 1–10% stained is scored as 1; 11–50% as 2; 51–80% as 3; and 81–100% as 4. Staining intensity is rate on a scale of 0 to 3 with 0 = negative, 1 = weak, 2 = moderate, and 3 = strong. The immunohistochemical score is calculated by adding the quantitative score and the intensity divided by 2. Therefore, theoretically, the scores could range from 0 to 6. A score of 3 to 4 was considered strong immunoreactivity.

Transfection and colony assays. For RNAi, A375 cells were seeded in 6-well plates at 3 x 10^5 cells/well and grown overnight to about 40% confluence prior to transfection. The empty BS/U6 RNAi vector (2 μg) or BS/U6-E2F1 RNAi vector together with neomycin (G418) resistance gene expression plasmid pcDNA-3 (0.2 μg) were cotransfected using Lipofectamine 2000 according to manufacturer's instructions. BS/U6 empty vector and BS/U6-E2F1 plasmid were a gift from Dr. Douglas Cress (The H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, Tampa, FL). After 48 hours, cells were plated at 1000 cells per 10 cm petri dish in RPMI containing 250 μg/ml G418. Transfections for each construct were plated in triplicate. After two weeks, the media was removed and individual colonies were stained with methylene blue solution (50% methanol and 0.5% methylene blue). The plates were rinsed with water and colony number enumerated.

RESULTS

E2F1 amplification in melanoma cells. Because we previously found increased copies of chromosome 20 in melanoma cell lines and the minimal region of cytogenetic alteration occurred at chromosome band 20q11,[4] we examined the E2F1 gene for amplification in human melanoma cells. Initially, we used FISH to analyze metaphase and interphase cells from a panel of 12 melanoma cell lines. As shown in (Fig. 1A), UACC 502 cell line had 6 copies of chromosome 20 and 6 copies of the E2F1 gene. The other eleven cell lines all had increased copies of the E2F1 gene (Table 1). The most notable abnormality identified by this technique was found in the cell lines UACC 647 and UACC 1022 where 6 to 9 copies of the E2F1 gene were observed in the cells. Our initial analysis of the malignant melanoma cell lines demonstrated extra copies of chromosome 20 in 14/15 samples. Four cell lines had previously been reported to have translocations of chromosomes 12 and 20.[4] The minimal region of cytogenetic alteration was found to occur at 20q11. Fluorescent in situ hybridization analysis of metaphase spreads isolated from 12 malignant melanoma cell lines hybridized with a labeled P1 clone for E2F1 demonstrated from 1 to 5 excess copies of E2F1 in 12/15 cell lines (Table 1). The E2F1 probe hybridized at the break-point of the translocation of chromosomes 12 and 20 in the cell lines UACC 2565, UACC 375, UACC 502 and UACC 903 (Fig. 1A). We next examined 12 different archival metastatic melanoma specimens. The stromal cells in these cases had two copies of E2F1. Only one of the metastatic melanoma lesions was diploid (Table 2). Eleven of the metastatic melanoma cases showed increased copies of the E2F1 gene (Fig. 1B). The extent of E2F1 amplification in the 12 archival metastatic melanoma specimens was not as great as was seen in the melanoma cell lines (Table 2).

To confirm the FISH results and to search for evidence of E2F1 gene rearrangements, we performed Southern blot analysis on *Bgl* II-digested DNAs extracted from 10 melanoma cell lines, as well as two control normal human melanocyte DNAs and normal dermal fibroblast DNA. As indicated in (Fig. 2), no rearrangements of the E2F1 were detected by this analysis. However, we did observe amplification of the E2F1 gene in several of the cell lines. The most notable copy number increase was seen in the cell lines UACC 612 and UACC 647.

E2F1 expression in melanoma cell lines. Western blot analysis was performed with lysates of several of the same cell lines used for the molecular cytogenetics studies. We used the KH20 and KH95 monoclonal antibodies

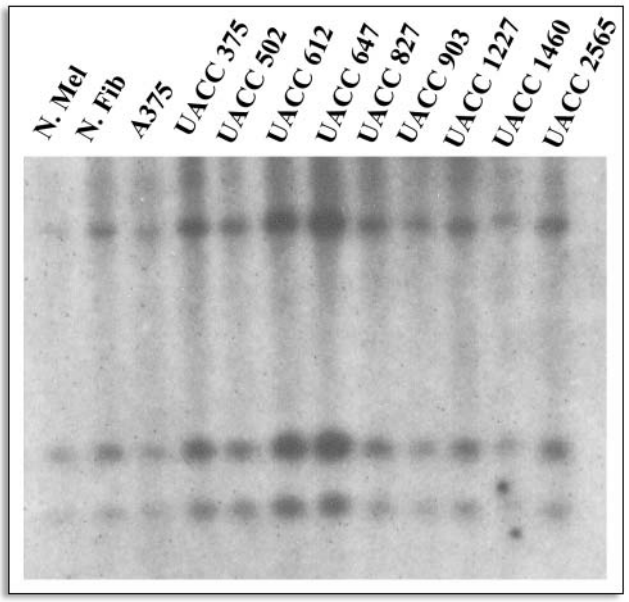

Figure 2. Southern Blot Analysis of E2F1 gene copy in melanoma cells. Genomic DNA was isolated from normal melanoma cell-lines. DNA samples were digested with *Bgl* II restriction enzyme and fractionated on 0.8% agarose gels. After agarose gel electrophoresis the DNA samples were transferred to a nylon membrane, hybridized with an E2F1 cDNA probe, and visualized by autoradiography.

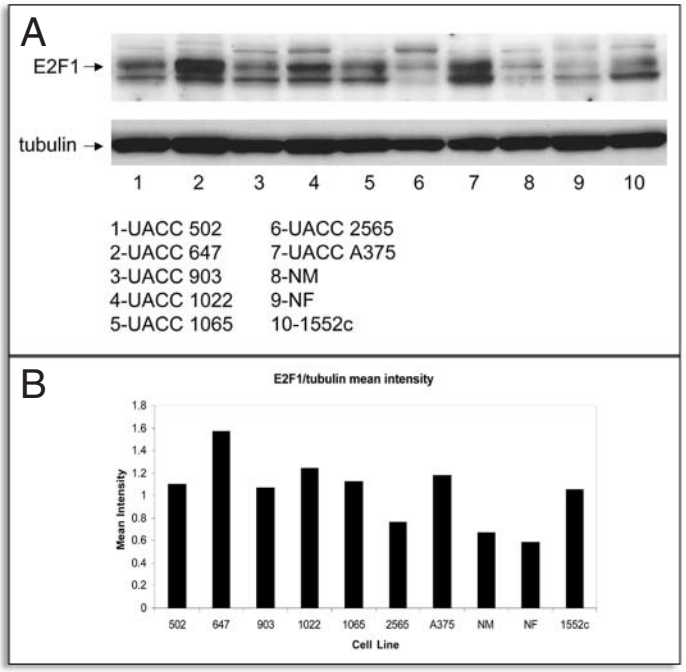

Figure 3. Detection of E2F1 protein in melanoma cells. Protein samples were extracted from normal melanocytes, normal fibroblasts and melanoma cells-lines. Proteins were resolved by gel electrophoresis and stained with anti-E2F1 antibody. The membranes were stripped and reprobed with anti-tubulin antibody after staining with E2F1 antibody for equal loading and protein integrity. The experiments were repeated at least twice.

for this series of experiments. The epitope for KH20 has been mapped to amino acids 1–89 of E2F1 and KH95 recognizes amino acids 342–386. The E2F1 protein was detected as a 55–60-kDa doublet. Relative to normal diploid fibroblasts and normal melanocytes, E2F1 expression was elevated in 7 out of 8 of the melanoma cell lines tested (Fig. 3). We detected nuclear and cytoplasmic E2F-1 protein in 13/15 different melanoma lymph node metastases by immunohistochemistry. Staining in the positive control was both nuclear and cytoplasmic. In contrast, normal melanocytes, lymphocytes, and fibroblasts were negative (Fig. 4). The staining was both cyotplasmic and nuclear. Both radial growth phase and vertical growth phase melanoma tumor tissue was positive.

Knockdown of E2F1 inhibits melanoma cell line growth. To directly demonstrate that reduced E2F1 protein level is responsible for the inhibition of A375 cell growth, we performed RNAi to knockdown endogenous E2F1 and examined its effect on cell growth by colony assay. A375 cells were transfected with RNAi empty vector or plasmid that express a small E2F1 hairpin RNA inhibitor. Endogenous E2F1 protein levels were efficiently and specifically reduced 48 hr after transfection as determined by Western blot analysis (Fig. 5A). The transfected cells were then subjected to colony assay. Colonies were scored after two weeks and a representative set of plates are shown in Figure 5B. Compared to the control cells, cells with reduced E2F1 levels formed significantly less colonies (Fig. 5C).

DISCUSSION

The incidence of malignant melanoma has tripled in the last three decades.[13] Chemotherapy, radiotherapy, and immunotherapy have produced limited success in patients. Once metastatic, the disease has very poor prognosis. Consequently, there is a need to identify important genetic alterations in melanoma development with the intent of developing new therapeutic modalities.

The Rb-E2F signaling pathway is an important regulator of cell cycle and differentiation and its dysfunction can lead to oncogenesis. E2Fs are the main cellular targets of Rb protein,[14] but Rb and E2F1 play opposite roles in the cell cycle: Rb-E2F complexes maintain cells in the G_1 phase until Rb is phosphorylated after a growth signal induces activation of cyclins/CDK4. Phosphorylated Rb releases E2Fs from the complex, whereupon accumulated E2Fs activate downstream genes such as DNA polymerase α, thymidine kinase 1 and 2, Dhfr, and cdc6 that induce cells to S phase.[15] Disruption of the Rb/E2Fs signaling pathway, for example, when the Rb is mutated, appears to play a critical role in cancer development and progression. In regards to melanoma, deregulated E2F1 transcriptional activity has been reported.[9] However, the molecular basis for these alterations remain to be elucidated.

In the present study, we observed increased copies of the E2F1 gene in malignant melanoma cell lines and in metastatic melanoma lesions. Similar findings have been observed in the HEL erythroleukemia cell line.[16] Gain of 20q and a low frequency of E2F1 gene amplification have also been observed in esophageal squamous cell carcinomas.[17] In regards to melanoma, our findings confirm and extend the observations of Okamoto et al.[18] These investigators used high resolution R-banding, comparative genomic hybridization, and c-DNA microarray analysis to demonstrate increased E2F1 copy number numbers results from cytogenetic aberration on chromosome 20 using melanoma cell lines. In the present study we demonstrate increased E2F1 copy number results in elevated E2F1 protein expression in melanoma cell lines as well as uncultured melanoma specimens.

Current models for E2F activity and function during the cell cycle suggest that its overexpression due to gene amplification might contribute to the growth advantage of melanoma cells. The activity of this transcription factor is tightly regulated during the G_1 phase of the cell cycle by inhibitory complexes of E2F and hypophosphorylated forms of pRb and pRB-related proteins such as p107 and

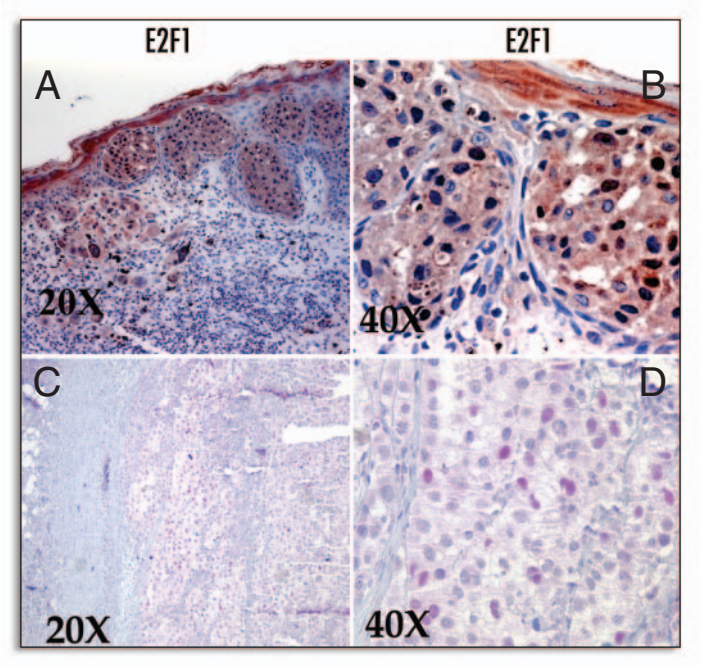

Figure 4. Immunohistochemical detection of E2F1. Metastic melanoma tumor sections were subjected to immunohistochemistry as described. (A) Representative E2F1 staining in a invasive primary melanoma ceases at 10x magnification. (B) Representative E2F1 staining of an invasive primary melanoma at 20x. The junctional nests of tumor as well the invasive dermal components are positive. Iimmunohistochemical stains original magnifications 20x and 40x. Note: the lymphocytes and fibroblasts serve as negative internal controls. (C) Representative E2F1 staining of a metastatic melanoma at 20x demonstrating nuclear staining. (D) Representative E2F1 staining at 40x demonstrating detail of the nuclear staining. Note: The lymphocytes and fibroblasts in the tissues serve as negative internal controls.

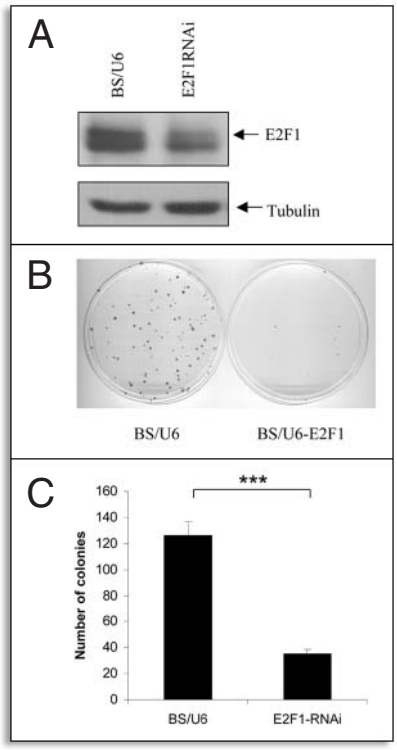

Figure 5. Inhibition of colony formation in E2F1 knockdown cells. (A) Reduced E2F1 expression in A375 cells transfected with E2F1 RNAi. Western blot analysis of total cell lysates of A375 cells transfected with BS/U6 or BS/U6-E2F1 for 48 hr. (B) Colony assay of A375 cells cotransfected with empty vector or E2F1 RNAi together with pcDNA-3. Transfected cells were grown for two weeks in the media containing 250 μg/ml G418. (C) Quantification of colonies in empty vector or E2F1 RNAi transfected cells. Results shown are from two experiments performed in triplicate. ***$p < 0.001$.

p130.[9] The available evidence suggests that free E2F normally is released when pRb is phosphorylated at the G_1/S phase transition and transactivates the expression of genes linked to cellular proliferation.[19] Increased levels of E2F expression due to gene amplification could abrogate the ability of pRb and other E2F-binding proteins to form inhibitory complexes during G_1 phase. The potential of E2F overexpression to override growth inhibitory signals has recently been shown experimentally by introducing the E2F1 cDNA into quiescent rodent cells.[20] Ectopic expression of E2F-1 also results in cellular transformation of rodent cells.[21,22] Furthermore, the constitutive expression of E2F1 in transgenic mice is associated with the development of squamous cell carcinomas of the skin[23] and conditional E2F1 activation leads to gonocyte-like dysplastic germ cells, resembling carcinoma in situ (CIS) in humans.[24] The physiologic significance of aberrant E2F1 expression as well as the identification of other proteins that work in conjunction with E2F1 to allow melanoma cells to proliferate will require further study.

These data have potential clinical ramifications. Genes regulated by E2F1 could be targets for anticancer agents.[25] For example, thymidylate synthase is an E2F-1 regulated enzyme that is essential for DNA synthesis and repair. Thymidylate synthase has long been identified as a potential target for cancer chemotherapeutic agents because of the essential role it plays in the de novo synthesis of thymidylate. More recently eugenol (4-Allyl-2-methoxyphenol) has been shown to inhibit melanoma growth in vitro and in vivo. Furthermore, the growth inhibitory effects of eugenol in melanoma cells was associated with a suppression of E2F1 transcriptional activity.[26] These two studies illustrate the opportunity to develop anticancer agents based on E2F biology.

In summary, we demonstrate amplification of the E2F1 gene in malignant melanoma. Increased copies of E2F1 lead to elevated expression of the E2F1 protein in melanoma cells and biopsy specimens. Previous work by Halaban and coworkers[9] implicate E2F in the autonomous growth of melanoma cells. Amplification of the E2F1 gene may explain the mechanism of the deregulation of the E2F1/pRB pathway. We propose that inhibition of E2F1 activity may be efficacious for the treatment of melanoma.

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