

Loss of SNF5 Expression Correlates with Poor Patient Survival in Melanoma

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Abstract **Purpose:** Aberrant expression of SWI/SNF chromatin remodeling complex is involved in cancer development. The tumor suppressor SNF5, the core subunit of SWI/SNF complex, has been shown to regulate cell differentiation, cell cycle control, and apoptosis. To investigate the role of SNF5 in the development of melanoma, we examined the expression of SNF5 in melanocytic lesions at different stages and analyzed the correlation between SNF5 expression and clinicopathologic variables and patient survival. **Experimental Design:** Using tissue microarray and immunohistochemistry, we evaluated SNF5 staining in 51 dysplastic nevi, 88 primary melanomas, and 48 metastatic melanomas. We studied chemosensitivity of melanoma cells with reduced SNF5 expression by siRNA using cell survival and apoptosis assays. **Results:** SNF5 expression was reduced in metastatic melanoma compared with dysplastic nevi ($P = 0.005$), in advanced primary melanoma (Clark's level V) compared with low risk Clark's level II melanoma ($P = 0.019$), and in melanoma at sun-exposed sites compared with sun-protected sites ($P = 0.044$). Furthermore, we showed a strong correlation between negative SNF5 expression and a worse 5-year survival in melanoma patients ($P = 0.016$). Multivariate Cox regression analysis revealed that negative SNF5 expression is an independent prognostic factor to predict patient outcome in primary melanomas ($P = 0.031$). Finally, we showed that knockdown of SNF5 in melanoma cell lines resulted in significant chemoresistance. **Conclusions:** Our data indicate that SNF5 may be an important marker for human melanoma progression and prognosis as well as a potential therapeutic target. (Clin Cancer Res 2009;15(20):6404–11)

Human cutaneous malignant melanoma is a life-threatening skin cancer, for its ability to metastasize rapidly and its resistance to conventional radiotherapy and chemotherapy (1, 2). Although melanoma accounts for only 4% of skin cancers, it is responsible for 80% of deaths from skin cancer (3). Furthermore, although melanoma is curable through early diagnosis and surgical excision (4), up to 20% of patients will develop metastatic tumor due to its highly invasive and metastatic properties (5). Consequently, metastatic melanoma patients

have a poor prognosis, with median survival of only 6 to 10 months and <5% of the patients surviving >5 years (4, 6, 7).

SWI/SNF ATP-dependent chromatin-remodeling complex is a 2-Mda multsubunit complex first identified in yeast and highly conserved among eukaryotes (8). SWI/SNF complexes play essential roles in transcriptional regulation, contribute to the control of cellular processes, such as proliferation and differentiation, and also are involved in DNA repair by altering the accessibility of UV-damaged DNA-binding proteins to DNA lesions (9–13). SNF5, also known as INI1/BAF47/SMARCB1, is the core subunit of mammalian SWI/SNF complex. Many genetic evidences have defined *SNF5* as a tumor suppressor gene in humans and mice. In mice, homozygous deletion of *SNF5* is embryonic lethal, and heterozygous mice are predisposed to develop tumors due to loss of heterozygosity for *SNF5* (14–16). In humans, homozygous inactivating mutations or deletions of the *SNF5* gene are associated with malignant rhabdoid tumors (MRTs) (17–19).

Studies have shown that *SNF5* exhibits an antiproliferative activity in MRTs because *SNF5* overexpression leads to a G_1 cell cycle arrest associated with an increase in p16INK4a, E2F, and Cyclin D (20–22). *SNF5* is also required for neural and adipocyte differentiation *in vitro* (23, 24). This suggests that *SNF5* is able to regulate the balance between cell proliferation and differentiation (24). Finally, the invasive property of malignant rhabdoid tumor is dramatically reduced upon *SNF5* expression

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Translational Relevance

Our study reveals that expression level of the tumor suppressor SNF5, the core subunit of SWI/SNF chromatin remodeling complex, is reduced in metastatic melanoma, indicating an important role of SNF5 in melanoma progression. We also show that negative SNF5 expression correlates with a worse 5-year melanoma patient survival compared with those of positive SNF5 expression. Consistent with this finding, we showed that melanoma cells depleted with SNF5 expression by RNA interference render cells resistant to chemotherapeutic drugs. The highly metastatic potential and resistance to conventional radiotherapy and chemotherapy are the major reasons for which melanoma patients succumb to the disease. Therefore, strategies to restore reduced SNF5 expression may be a potential approach for melanoma therapy. Moreover, analyzing SNF5 expression in melanoma patients may predict the response to chemotherapy.

in a RhoA-dependent manner (25). In contrast, inactivation of SNF5 caused murine embryonic fibroblasts and HeLa cells to undergo G₁ cell cycle arrest, followed by a p53-dependent apoptotic response (26, 27).

A number of immunohistochemistry studies focused on the ATPase subunit of SWI/SNF complex, BRG1 and BRM. Reduced BRG1 and BRM expression was found in breast, lung, and prostate cancer cell lines, and associated with poor prognosis for the patients with non-small cell lung cancer (28–33). However, few studies have focused on SNF5, the core subunit of SWI/SNF complex, in cancers. Although similar phenotypes of *SNF5* and *BRG1* knockout mice might suggest that both genes function in partially redundant pathways, it has been shown that loss of *SNF5* does not affect the expression of BRG1 target genes or the assembly of SWI/SNF complex (34). This indicates that SNF5 and BRG1 might not have the exact same functions. To investigate the role of SNF5 in the development of melanoma, we used tissue microarray (TMA) technology and immunohistochemistry to evaluate SNF5 expression in different stages of human melanocytic lesions. Our data indicate that reduced SNF5 expression is significantly associated with melanoma progression and a worse patient survival. We also found that SNF5 is an independent prognostic factor for human melanoma. Furthermore, we showed that downregulation of SNF5 protein level in melanoma cell lines enhanced chemoresistance of melanoma cells.

Materials and Methods

Construction of TMA. Formalin-fixed, paraffin-embedded tissues from 51 dysplastic nevi, 88 primary melanomas, and 48 metastatic melanomas were used for this study. Five cases of normal skin tissues and five cases of normal nevi were included as positive controls. All specimens were obtained from the 1990 to 1998 archives of the Department of Pathology, Vancouver General Hospital. The use of human skin tissues was approved by the medical ethical committee of the University of British Columbia and was done in accordance with the Declaration

of Helsinki guidelines. The most representative tumor area was carefully selected and marked on the H&E-stained slide. The TMAs were assembled using a tissue-array instrument (Beecher Instruments). Duplicate 0.6-mm-thick tissue cores were taken from each biopsy specimen. Two composite high-density TMA blocks containing 126 and 111 cases from a total of 237 patients were designed. Using a Leica microtome (Leica Microsystems, Inc.), multiple 4- μ m sections were cut and transferred to adhesive-coated slides using regular histology procedures. H&E were used to stain one section from each TMA. Other sections were kept at room temperature for immunohistochemical staining.

Immunohistochemistry of TMA. TMA slides were dewaxed at 55°C for 20 min followed by three 5-min washes with xylene. The rehydration of tissues was done by 5-min washes in 100%, 95%, and 80% ethanol and distilled water. Antigen retrieval was done by heating the samples at 95°C for 30 min in 10 mmol/L sodium citrate (pH 6.0). Endogenous peroxidase activity was blocked by incubation in 3% hydrogen peroxide for 30 min. After 30 min of blocking with the universal blocking serum (Dako Diagnostics), the sections were incubated with monoclonal mouse anti-SNF5 antibody (1:200 dilution; Abcam) at 4°C overnight. The sections were then incubated for 30 min each with a biotin-labeled secondary antibody and then streptavidin-peroxidase (Dako Diagnostics). The samples were developed using 3,3'-diaminobenzidine substrate (Vector Laboratories) and counterstained with hematoxylin. Dehydration was then done following a standard procedure and the slides were sealed with coverslips. Negative controls were done by omitting the SNF5 antibody during the primary antibody incubation.

Evaluation of immunostaining. The evaluation of SNF5 staining was examined by one dermatopathologist and two other observers simultaneously, and a consensus was reached for each core. Microarray

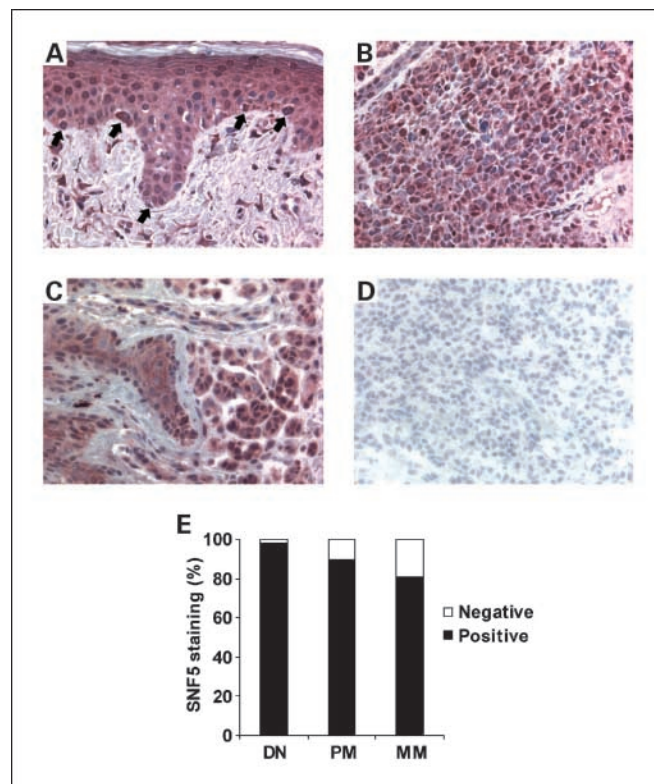


Fig. 1. Correlation between SNF5 expression and melanoma progression. A to D, representative images of SNF5 immunohistochemical staining. A, positive staining in normal skin (arrows, melanocytes); B, positive staining in benign nevi; C, positive SNF5 staining in dysplastic nevi; and D, negative SNF5 staining in primary melanoma. Magnification, $\times 400$. E, reduced SNF5 expression correlates with melanoma progression. A significant difference of SNF5 staining was observed between dysplastic nevi (DN) and metastatic melanoma (MM; $P = 0.005$, χ^2 test).

positivity for SNF5 was defined as any detectable nuclear or cytoplasmic staining (35). Examples for positive and negative SNF5 staining are given in Fig. 1C and D.

Cell culture and transfection. MMRU and MEWO human melanoma cell lines were cultured in DMEM supplemented with 10% fetal bovine serum (Invitrogen). All cells were maintained in 5% CO₂ atmosphere at 37°C. Cells were grown to 50% confluency before small interfering RNA (siRNA) transfection. Nonspecific control siRNA or SNF5 siRNA (Qiagen) was transfected by siLentFect Lipid Reagent (Bio-Rad) according to the manufacturer's instructions. Twenty-four hours after transfection, the medium containing transfection reagents was removed. The cells were rinsed twice with PBS and incubated in fresh medium. Forty-eight hours after transfection, cells were lysed for Western blot assay, and subjected to sulforhodamine B (SRB) cell survival assay and apoptosis analysis.

Western blot analysis. Cells were harvested and washed thrice with PBS. Whole cell proteins were extracted as described previously (36). Protein concentrations were determined by protein assay (Bio-Rad). Western blot analysis was done as described previously (37). The following antibodies were used for Western blot: mouse anti-SNF5 (Abcam) and mouse anti-β-actin (1:1,000; Sigma). IR IRDye-labeled secondary antibody was applied to the blot for 1 h at room temperature. The signals were detected with Odyssey IR Imaging system (LI-COR).

SRB cell survival assay. Cells were transfected with SNF5 siRNA for 48 h, and then treated with various doses of doxorubicin, etoposide, and camptothecin (Sigma). Twenty-four hours after drug treatments, cells were fixed with 10% trichloroacetic acid at 4°C for 1 h, and the cell survival was quantitated by SRB staining (38).

Apoptosis analysis. Cells were transfected with SNF5 siRNA for 48 h and then treated with 0.25 μg/mL doxorubicin, 5 μmol/L etoposide, and 25 nmol/L camptothecin. Twenty-four hours after drug treatments, cells were fixed with 70% ethanol at 4°C for 1 h, and cells were stained with 40 μg/mL propidium iodide in hypotonic fluorochrome buffer (0.1% Triton X-100, 0.1% sodium citrate, and 25 μg/mL RNase A) for 30 min. Samples were then analyzed using a FACSCanto flow cytometer (BD Biosciences).

Statistical analysis. For TMA, statistical analysis was done with SPSS 11.5 software (SPSS). The χ² test was used to compare the quantitative differences of SNF5 staining in different stages of melanoma progression. The association between SNF5 staining and the clinicopathologic parameters of the primary melanoma patients, including age, gender, tumor thickness, ulceration, histologic subtype, location, and Clark's level, was also evaluated by χ² test. The Kaplan-Meier method and log-rank test were used to evaluate the correlation between SNF5 expression and patient survival. Cox regression model was used for multivariate analysis. For SRB cell survival and apoptosis assays, student's *t* test was used. A *P* value of <0.05 was considered significant.

Results

Clinicopathologic features of TMAs. Fifty-one cases of dysplastic nevi, 88 cases of primary melanomas, and 48 cases of metastatic melanomas are evaluated for SNF5 staining. As shown in Table 1, for the 88 cases of primary melanomas, there were 44 males and 44 females, with age ranging from 21 to 93 years (median, 57 years). For primary melanoma staging, Breslow thickness and Clark's level were used as criteria for evaluating SNF5 expression: 26 tumors were ≤1.0 mm, 30 tumors were 1.01 to 2.0 mm, 14 tumors were 2.01 to 4.0 mm, and 18 were >4.0 mm; 20 tumors were at Clark's level II, 25 tumors were at level III, 31 tumors were at level IV, and 12 tumors were at level V. Ulceration was observed in 18 cases. For the histologic subtype, 13 tumors were nodular melanomas, 39 tumors were superficial spreading melanomas, 15 tumors were lentigo malig-

Table 1. SNF5 staining and clinicopathologic characteristics of 88 primary melanomas

Variables	SNF5 staining			P*
	Negative	Positive	Total	
Age				
≤57	3 (7%)	41 (93%)	44	0.291
>57	6 (14%)	38 (86%)	44	
Gender				
Male	7 (13%)	47 (87%)	54	0.286
Female	2 (6%)	32 (94%)	34	
Tumor thickness (mm)				
≤1.0	2 (8%)	24 (92%)	26	0.288 [†]
1.01-2.0	2 (7%)	28 (93%)	30	
2.01-4.0	2 (14%)	12 (86%)	14	
>4.0	3 (17%)	15 (83%)	18	
Ulceration				
Present	1 (6%)	17 (94%)	18	0.463
Absent	8 (11%)	62 (89%)	70	
Tumor subtype				
Nodular	0 (0%)	13 (100%)	13	0.187 [‡]
Superficial spreading	3 (8%)	36 (92%)	39	0.484 [‡]
Lentigo maligna	3 (20%)	12 (80%)	15	0.170 [‡]
Unspecified	3 (14%)	18 (86%)	21	0.482 [‡]
Site [§]				
Sun-protected	5 (7%)	66 (93%)	71	0.044
Sun-exposed	4 (24%)	13 (76%)	17	
Clark's level				
II	0 (0%)	20 (100%)	20	0.019
III	3 (12%)	22 (88%)	25	
IV	3 (10%)	28 (90%)	31	
V	3 (25%)	9 (75%)	12	

*χ² test.

[†]Comparison of thickness ≤2 and >2 mm.

[‡]Comparison of the specified group with all the other groups.

[§]Sun-protected sites: trunk, arm, leg and feet; sun-exposed sites: head and neck.

^{||}Comparison of Clark's level II and V.

na melanomas, and 21 tumors were nonspecified. Seventeen melanomas were located in sun-exposed sites (head and neck), and 71 were located in sun-protected sites (other locations).

Reduced SNF5 expression correlates with melanoma progression. To investigate if SNF5 expression is changed in pigmented melanocytic lesions, immunohistochemistry staining of dysplastic nevi, primary melanoma, and metastatic melanoma were done using TMA technique (Fig. 1). Positive SNF5 staining was recorded in 98%, 90%, and 81% of the biopsies in dysplastic nevi, primary melanoma, and metastatic melanoma, respectively (Fig. 1E). Melanocytes in all five cases of normal skin tissues and five benign nevi showed strong positive SNF5 staining (Fig. 1A and B). Although the majority of the biopsies from different stages of melanocytic lesions have positive staining, negative SNF5 staining was significantly increased in metastatic melanoma when compared with dysplastic nevi (*P* = 0.005, χ² test). However, there is no significant difference in SNF5 staining between dysplastic nevi and primary melanoma (*P* = 0.069, χ² test), or between primary melanoma and metastatic melanoma (*P* = 0.161, χ² test).

Reduced SNF5 expression correlates with Clark's level and tumor location. Because Clark's level of invasion is an important prognostic marker for patients with primary melanoma, we first studied if SNF5 expression correlates with Clark's level. We found a significant difference in SNF5 expression between

Clark's level V and Clark's level II ($P = 0.019$, χ^2 test; Fig. 2A). All the tumors in Clark's level II had positive SNF5 staining, whereas only 75% of tumors in Clark's level V had positive SNF5 expression.

As UV radiation is the main environmental factor for melanoma formation, we analyzed the SNF5 staining in sun-exposed or sun-protected sites. Reduced positive SNF5 staining significantly correlated with the location of primary melanomas ($P = 0.044$, χ^2 test; Fig. 2B). Although 93% of tumors from sun-protected sites (trunk, arm, leg, and feet) had positive SNF5 staining, SNF5 staining was reduced to 76% in tumors from sun-exposed sites (head and neck). We did not find significant correlations between SNF5 expression with other clinicopathologic variables, including Breslow tumor thickness, subtype, ulceration, or patient's age or gender (Table 1).

Reduced SNF5 expression correlates with poor patient survival. To evaluate whether reduced SNF5 staining in human primary and metastatic melanomas correlate with a worse prognosis, Kaplan-Meier survival curves were constructed using overall or disease-specific 5-year survival to evaluate the biopsies with positive SNF5 staining to those with negative SNF5 staining. Our data revealed that positive SNF5 staining correlated with both overall and disease-specific 5-year survival in primary melanomas ($P = 0.016$ and $P = 0.049$, respectively, log-rank test; Fig. 3A and B). The correlation is also significant

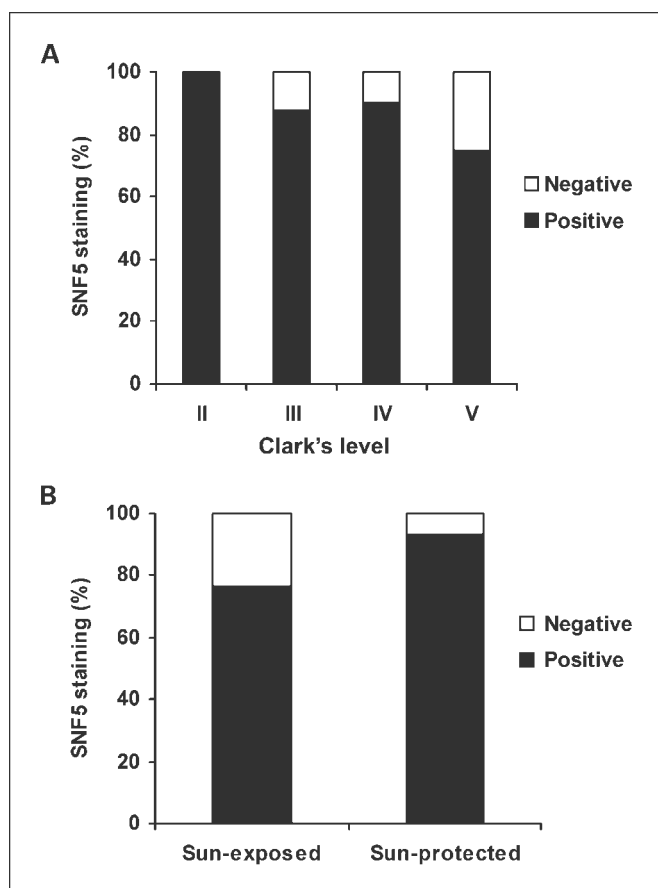


Fig. 2. Correlation between SNF5 expression and other clinicopathologic parameters in primary melanoma. **A**, reduced SNF5 expression is correlated with Clark's level ($P = 0.019$, χ^2 test, comparison between level II and V). **B**, reduced SNF5 expression is correlated with sun-exposed tumor sites ($P = 0.044$, χ^2 test).

when primary and metastatic melanoma cases are combined for the analysis; both overall and disease-specific 5-year survival rates are significantly better for patients with positive SNF5 expression in their tumor tissues compared with those with negative SNF staining ($P = 0.029$ and $P = 0.040$, respectively, log-rank test; Fig. 3C and D).

In addition, we examined whether positive SNF5 expression is an independent prognostic marker for melanoma. We performed a multivariate analysis including SNF5 expression, age, gender, tumor thickness, ulceration, location, and subtype for 88 primary melanomas. We found that similar to tumor thickness and presence of ulceration, which have been widely accepted as independent prognostic factors for melanoma patient survival, SNF5 expression is also an independent prognostic factor for both overall [relative risk, 5.145; 95% confidence interval (CI), 1.48-17.89; $P = 0.010$; Table 2] and disease-specific 5-year survival (relative risk, 4.637; 95% CI, 1.15-18.63; $P = 0.031$; Table 2). Our results clearly indicate that negative SNF5 expression in either primary or metastatic melanoma is associated with poor prognosis, suggesting that SNF5 reduction may serve as a molecular prognostic marker for this aggressive disease.

Resistance to chemotherapeutic drugs increases in SNF5 knock-down melanoma cell lines. A major obstacle in treating melanoma is its resistance to drug-induced apoptosis (39). Because negative SNF5 expression strongly correlates with poor patient survival, we investigated the involvement of SNF5 in chemo-resistance of melanoma cells. We first transiently transfected MMRU and MEWO melanoma cells with SNF5 siRNA or control siRNA. Forty-eight hours after transfection, cells were harvested for Western blot analysis (Fig. 4A) or subjected to cell survival and apoptosis assays (Fig. 4B and C). Western blot indicated that at least 75% knockdown of SNF5 protein expression in both MMRU and MEWO cells transfected with SNF5 siRNA compared with those transfected with control siRNA. In SRB cell survival assay, cell survival of both MMRU and MEWO SNF5 knockdown cells treated with doxorubicin, etoposide, and camptothecin was significant higher than those of control cells (Fig. 4B). However, we did not observe a significant difference in cell proliferation upon SNF5 knockdown in both MMRU and MEWO cells when compared with the control cells without drug treatment (Supplementary Fig. S1). To investigate if reduced chemosensitivity of SNF5 knockdown cells is due to reduced apoptosis, we performed flow cytometry analysis. Our data showed that both drug-treated MMRU and MEWO SNF5 knockdown cells have significantly lower sub-G1 populations compared with control cells (Fig. 4C). Thus, these data indicate that downregulation of SNF5 expression reduces drug-induced apoptosis in melanoma cells. This might suggest that melanoma resistance to chemotherapy is at least partially due to the reduction of SNF5 protein level.

Discussion

Several lines of evidence indicated that aberrant expression of the SWI/SNF chromatin remodeling complex proteins are involved in tumorigenesis. Deletion or mutation of the *BRG1* gene was found in lung, breast, prostate, and melanoma cancer cell lines (28, 30, 31, 33, 40). The *SNF5* gene was also found inactivated in MRTs and downregulated in epithelioid sarcomas

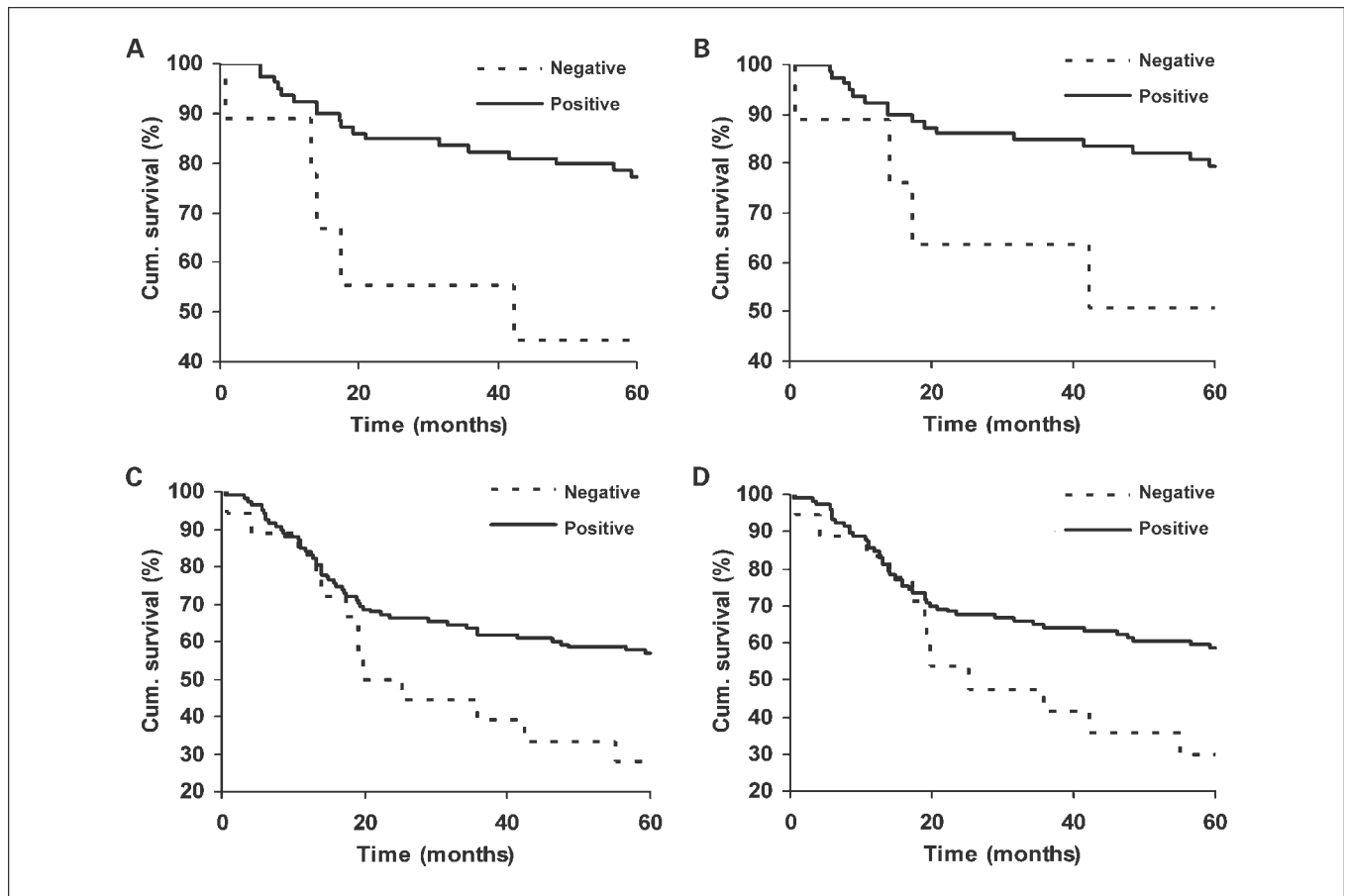


Fig. 3. Kaplan-Meier survival analyses of melanoma patients. Patients with negative SNF5 expression have a significantly worse 5-y survival than those with positive SNF5 expression. *A*, overall survival for primary melanoma patients ($P = 0.016$, log-rank test). *B*, disease-specific survival for primary melanoma patients ($P = 0.041$, log-rank test). *C*, overall survival for all melanoma patients, (including primary and metastatic cases; $P = 0.029$, log-rank test). *D*, disease-specific survival for all melanoma patients ($P = 0.040$, log-rank test). *Cum.*, cumulative.

(25, 41). Our result reveals a significant correlation between reduced SNF5 expression and melanoma progression (Fig. 1E). However, our finding is in contrast with the report by Hornick et al. (41) showing SNF5 expression is intact in metastatic melanoma. We argue that the discrepancy may be due to lack of proper control and the smaller sample size in their study. They tested only 20 cases of metastatic melanomas without compar-

ison to nevi or primary melanoma biopsies. Therefore, it is inaccurate for these authors to claim that SNF5 expression is indeed intact in metastatic melanoma. Different antibodies used in the immunohistochemical studies could also be the cause of the discrepancy.

Although the difference in SNF5 expression between dysplastic nevi and primary melanoma only reaches a borderline

Table 2. Cox regression analysis of SNF5 expression and other clinicopathologic characteristics on 5-y survival of 88 primary melanoma patients

Variable*	Overall survival		Disease-specific survival	
	Relative risk (95% CI)	P	Relative risk (95% CI)	P
SNF5	5.145 (1.48-17.89)	0.010	4.637 (1.15-18.63)	0.031
Age	0.775 (0.28-2.12)	0.620	0.870 (0.29-2.57)	0.802
Gender	3.215 (1.29-8.00)	0.012	2.804 (1.06-7.44)	0.038
Thickness	0.296 (0.09-0.94)	0.039	0.233 (0.06-0.87)	0.030
Ulceration	0.293 (0.10-0.85)	0.024	0.243 (0.08-0.76)	0.015
Location	0.705 (0.26-1.93)	0.497	0.601 (0.21-1.71)	0.340
Subtype	0.476 (0.13-1.81)	0.276	0.531 (0.14-2.07)	0.362

*Coding of variables: SNF5 was coded as 1 (negative), and 2 (positive). Age was coded as 1 (≤ 57 y), and 2 (> 57 y). Gender was coded as 1 (female) and 2 (male). Thickness was coded as 1 (≤ 2 mm), and 2 (> 2 mm). Ulceration was coded as 1 (absent) and 2 (present). Location was coded as 1 (head and neck), and 2 (others). Subtype was coded as 1 (nodular) and 2 (others).

significance (Fig. 1E), which might be due to insufficient sample size ($P = 0.069$, χ^2 test), a trend of decreased SNF5 staining in primary melanoma was observed (2% negative SNF5 staining in dysplastic nevi versus 10% in primary melanoma). This suggests a possible role of SNF5 in the initiation of melanoma. It is also worth to note that reduced SNF5 expression correlates

with tumor location at sun-exposed sites (Fig. 2B), indicating a crucial role of UV radiation in regulating SNF5 level. Reduction of SNF5 level could be due to UV-induced mutation at the SNF5 locus at 22q11.2, which is found homozygously deleted or mutated in MRTs (12). Besides the role of SNF5 in melanoma initiation, SNF5 has been shown to play a role during

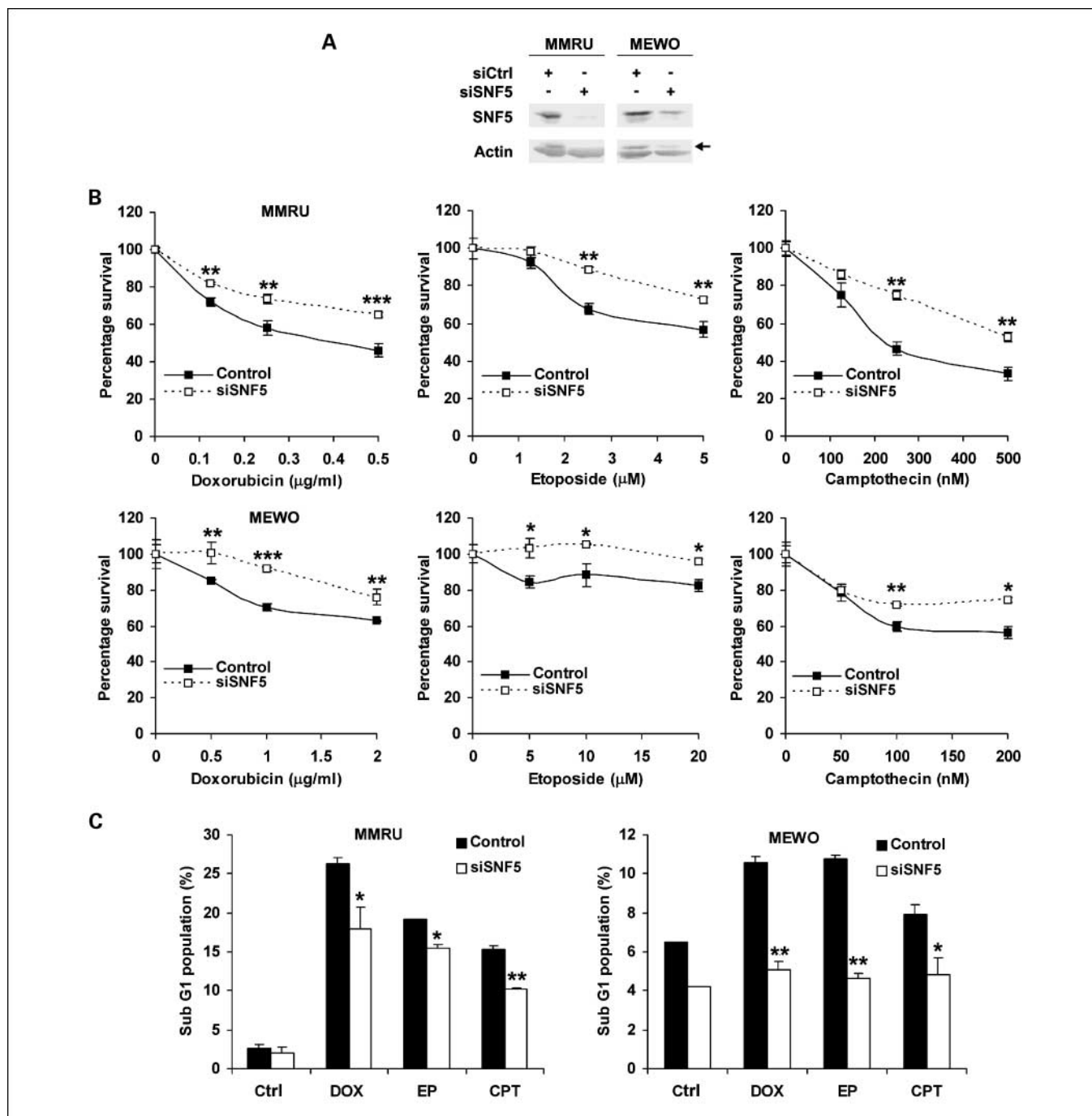


Fig. 4. Chemotherapeutic drug-induced apoptosis is repressed in SNF5 knockdown melanoma cells. **A**, SNF5 protein knockdown level detected by Western blot (arrow, SNF5 band from previous blot). **B**, drug-induced cell survival by SRB staining. Forty-eight hours after transfection with SNF5 siRNA or control siRNA, MMRU and MEWO cells were treated with doxorubicin, etoposide, or camptothecin. Twenty-four hours after drug treatments, cells were fixed with 10% trichloroacetic acid for 1 h and quantitated by SRB staining. **C**, drug-induced apoptosis assayed by fluorescence-activated cell sorting. Forty-eight hours after transfection with SNF5 siRNA or control siRNA, MMRU and MEWO cells were treated with 0.25 $\mu\text{g/ml}$ doxorubicin (DOX), 5 $\mu\text{mol/L}$ etoposide (EP), and 25 nmol/L camptothecin (CPT) for 24 h, or without drug treatment as control (Ctrl). Cells were then stained with 40 $\mu\text{g/ml}$ propidium iodide for 30 min, and the percentage of apoptotic (sub-G1) cells was measured by flow cytometry. All experiments were carried out in triplicate. Columns, mean; bars, SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

nucleotide excision repair pathway, which repairs UV-induced DNA photolesions (9). In yeast, SNF5 is found to interact with Rad4, a DNA-damaged recognition protein, to enhance nucleotide excision repair after UV irradiation (42). Therefore, reduced SNF5 expression may enhance accumulation of mutations and genomic instability that drive the subsequent progression of the disease.

In agreement with the correlation between reduced SNF5 expression and melanoma progression, reduced SNF5 expression also correlates with overall and disease-specific 5-year patient survival (Fig. 3), and is likely an independent factor predicting patient outcome (Table 2). Although the difference in SNF5 expression between primary melanoma and metastatic melanoma did not reach significance ($P = 0.161$, χ^2 test), a linear trend of decreased SNF5 staining in metastatic melanoma was still observed (negative SNF5 staining in 10% primary melanomas compared with 19% in metastatic melanomas). Furthermore, SNF5 expression was reduced significantly in Clark's level V compared with level II. These stage-specific expression patterns suggest that reduced SNF5 activity might be required for the progression from primary tumors to metastatic tumors. We did not find statistical difference in the correlation between reduced SNF5 expression and Breslow tumor thickness ($P = 0.288$, χ^2 test). Invasiveness of cancer cells is defined by their ability to migrate and invade through extracellular matrix and neighboring cells (43). In fact, the role of SWI/SNF in melanoma cell migration and invasion is not clear. One study showed that overexpression of SNF5 reduces cell migration properties in a RhoA-dependent manner in malignant rhabdoid tumor cells (25), whereas Sun et al. (32) showed that overexpression of BRG1 and BRM enhances prostate cancer cell invasion. Therefore, more studies are required on the role of SNF5 in melanoma cell invasion, for instance, whether SNF5 regulates the expression and activity of matrix metalloproteinases.

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Acquired resistance to apoptosis is a hallmark of cancer (44), which allows cancer cells to escape drug-induced apoptosis and enables the establishment of metastasis. Metastatic melanoma is particularly resistant to conventional radiotherapy and chemotherapy (1, 2). A study by Oh et al. (45) indicating that doxorubicin-induced apoptosis is repressed in NIH3T3 cells transduced with dominant-negative form of BAF60a. BAF60a, a mediating subunit of SWI/SNF complex proteins, interacts with p53, and uncoupling of p53 with the SWI/SNF complex resulted in repression of apoptosis and cell cycle arrest. Because melanomas rarely harbor p53 mutations (46, 47), SNF5 might play a role in regulating apoptosis in melanoma cells. Indeed, our *in vitro* data revealed that knocking down SNF5 in melanoma cells with siRNA triggered stronger resistance to chemotherapeutic drugs (Fig. 4). Combined with patient survival data, this finding led us to speculate that reduced SNF5 expression may contribute to chemoresistance and thus decreased survival in melanoma patients. Nevertheless, the molecular mechanisms of SNF5 and its relations to BAF60a in apoptosis of melanoma cells after chemodrug treatment warrant further investigation.

In conclusion, the data from this study show that SNF5 expression is reduced in human cutaneous melanoma and significantly correlated with patient survival, suggesting that SNF5 plays an important role in melanomagenesis and it may serve as a promising prognostic marker and the therapeutic target for malignant melanoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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