Melanoma is among the most malignant cancers with notorious aggressiveness, and its prognosis is greatly influenced by progression status. Serum microRNAs are small noncoding RNAs with high stability and easy accessibility in human blood. Their expression profiles are frequently dysregulated in cancers; hence, levels of serum microRNAs may reflect progression status and thus predict melanoma prognosis. In a hospital based case-control study, we found a significant reduction of serum miR-16 level in melanoma patients compared with cancer-free controls \( (P < 0.001) \). In addition, serum miR-16 level markedly decreased in melanoma patients with increased tumor thickness, occurrence of ulceration, and advanced American Joint Committee on Cancer stages, and was highly correlated with tissue Ki-67 expression \( (r = -0.521, P < 0.0001) \). Kaplan-Meier analysis and Cox proportional hazards regression analysis revealed a prognostic role of serum miR-16 (hazard ratio 2.49, 95% confidence interval 1.10–5.63, \( P = 0.028 \)), which independently evaluated patients’ survival outcome. Finally, the suppressive role of miR-16 in melanoma growth was validated both in vitro and in vivo. In conclusion, we demonstrated that serum miR-16 is a potential biomarker for predicting melanoma prognosis.


**INTRODUCTION**

Melanoma is notoriously aggressive malignancy, with one of the most increasing incidences of all types of cancers in the United States in 2015 (Siegel et al., 2015). The classification of American Joint Committee on Cancer (AJCC) stratified melanoma patients into four prognostic groups with optimal scheme of treatment according to the progression status (Balch et al., 2009). Generally, melanoma patients at AJCC stage I or II can be effectively cured by surgical resection. However, for patients at AJCC stage IV, the prognosis is extremely poor, with a 10-year survival rate <20% (Balch et al., 2009). Undoubtedly, establishment of biomarkers for efficient evaluation of progression and prognosis will contribute much to the medical management of melanoma patients.

Several histopathologic features have long been used by the AJCC staging system in evaluating melanoma progression and prognosis, including tumor thickness, ulceration status, and mitotic activity (Hill et al., 2015). Unexpectedly, a small subgroup of thin, nonulcerated, nonmitotically active melanomas that could not be identified by any of these features are rather aggressive, probably because of their special genetic heterogeneity (Abbas et al., 2014; Lo and Fisher, 2014).

Recently, some potentially applicable molecular biomarkers, such as Ki-67, proliferating cell nuclear antigen, and matrix metalloproteinase 2, have been revealed to be of independent prognostic significance (Gould Rothberg et al., 2009; Schramm and Mann, 2011). However, some contradictory conclusions about these molecules in immunohistochemistry staining analyses hinder their application in evaluating patient prognosis (Frahm et al., 2001; Hazan et al., 2002; Ostmeier et al., 2001; Sparrow et al., 1998). Moreover, there are inevitable biases in the measurements of these previously described histopathologic features and molecular biomarkers. For example, tumor thickness can be influenced by epidermal hyperplasia and the involvement of skin appendages, both of which may lead to a spuriously thicker estimate (Elder, 2015). Therefore, new biomarkers with less invasiveness, greater feasibility, and higher methodological stability are needed to improve the sufficiency and accuracy in predicting melanoma prognosis.

MicroRNAs (miRNAs) are an abundant class of small noncoding RNAs that negatively regulate target gene expressions at the posttranscriptional level (Su et al., 2015). The expression profiles of miRNAs are frequently dysregulated during the progression of carcinogenesis, suggesting their important roles in cancer initiation and development (Garzon et al., 2009; Lin and Gregory, 2015; Lu et al., 2005; Takahashi et al., 2015; Zaravinos, 2015). More importantly, it is reported that miRNAs are present in the peripheral circulation, even under some extreme circumstances (Chen et al., 2008; Mitchell et al., 2008). Because of the stability in human blood, the facility of blood sampling, and the standardized PCR-based assays, serum miRNAs have emerged as valuable biomarkers of progression and prognosis in different cancers (Mitchell et al., 2008; Ng et al., 2009; Resnick et al., 2009; Wu et al., 2011). As for melanoma, several serum miRNAs, such as miR-206, miR-211, and...
miR-125b, have been reported to be associated with progression and prognosis (Alegre et al., 2014; Fleming et al., 2015; Friedman et al., 2012; Kanemaru et al., 2011; Tian et al., 2015), suggesting the high potential of serum miRNAs as prognosticators for melanoma patients.

Herein, through an miRNA microarray study, we found that serum miR-16 level was significantly decreased in melanoma patients compared with the cancer-free controls. We then analyzed the associations of serum miR-16 level with tumor thickness, ulceration status, AJCC stage, and tissue Ki-67 expression, respectively, to evaluate its potential in determining the progression status. Kaplan-Meier analysis, Cox proportional hazards regression analysis, and stratified analysis were subsequently performed to assess the prognostic role of serum miR-16 in melanoma. Finally, the effect of dysregulated miR-16 expressions on melanoma was investigated both in vitro and in vivo.

RESULTS

Significant reduction of serum miR-16 level in melanoma patients

To identify the expression profiles of serum miRNAs in melanoma patients, we first performed a miRNA microarray study using serum samples obtained from 20 melanoma patients and 20 matched cancer-free controls. We found that 40 miRNAs showed at least a threefold change in serum level between patients and controls, among which 21 miRNAs displayed more than a threefold decrease, including miR-16 (see Supplementary Table S1 online). Since the first discovery in chronic lymphocytic leukemia, miR-16 has been reported to act as tumor suppressors or potential onco-miRs in different types of cancers (Huang et al., 2015). We considered that miR-16 was a cancer-associated miRNA and may play important roles in melanoma as well.

We then validated serum miR-16 level by quantitative real-time PCR in a cohort consisting of 120 melanoma patients and 120 cancer-free controls. Consistent with the results of the microarray study, serum miR-16 level was significantly decreased in melanoma patients compared with controls ($P < 0.001$; Figure 1a). The capacity of serum miR-16 level to discriminate melanoma patients from controls was then tested by a receiver operating characteristic curve with an area under the curve value of 0.779. With a cutoff value of 0.585, sensitivity and specificity were 80.0% and 71.7%, respectively (Figure 1b). We further investigated whether serum miR-16 levels could be used to evaluate melanoma progression and prognosis.

Association between serum miR-16 level and melanoma patient characteristics and AJCC stages

We collected the characteristics information of the 120 melanoma patients. There were 30 patients in each of the AJCC stages, and slightly more women than men (55.8% vs. 44.2%). Mean patient age was 51.59 years (standard deviation 14.38 years) at diagnosis. Information on tumor thickness, ulceration status, and tumor site was also collected (see Supplementary Table S2 online). Median follow-up time was 39.32 months, during which 38 patients died by the last follow-up.

We then analyzed the association between serum miR-16 level and melanoma patient characteristics. Analysis revealed that serum miR-16 level was significantly decreased in patients with tumor thickness $>1$ mm vs $\leq 1$ mm ($P = 0.002$). Serum miR-16 levels declined markedly in patients with ulceration than in those without ($P = 0.011$), whereas no significant difference was found in terms of age, sex, or tumor site (see Supplementary Table S2). Moreover, analysis of the 2015 TCGA Skin Cutaneous Melanoma dataset revealed that tissue miR-16 expressions were significantly correlated with tumor thickness ($r = -0.143$, $P = 0.0083$; see Supplementary Figure S1 online). Tissue miR-16 expressions were significantly decreased in patients with tumor thickness $\geq 2$ mm versus $<2$ mm ($P = 0.0023$; see Supplementary Table S3 online). Because tumor thickness and ulceration status are two crucial clinicopathologic factors determining melanoma progression (Dickson and Gershenwald, 2011), our findings implied that serum miR-16 levels could reflect progression status of melanoma patients.

Analysis also revealed that serum miR-16 level remarkably decreased in patients at advanced stages (AJCC stages III and IV vs. early AJCC stages I and II, $P < 0.001$; see Supplementary Table S2). Specifically, serum miR-16 level significantly decreased in patients at AJCC stages III and IV compared with those at AJCC stage I ($P < 0.05$.

Figure 1. Significant reduction of serum miR-16 level in melanoma patients. (a) Levels of serum miR-16 in 120 melanoma patients and 120 cancer-free controls. miR-16 level was normalized to Cel-miR-39 in serum. (b) Generated receiver operating characteristic curve of serum miR-16 had an area under the curve (AUC) value of 0.779. Using a cutoff value of 0.585, sensitivity and specificity were 80.0% and 71.7%, respectively, in distinguishing melanoma patients from cancer-free controls. Two-tailed Student t test was used to analyze significant differences. ***$P < 0.001$. 

Journal of Investigative Dermatology (2016), Volume 136, 986
scores similarly were significantly correlated (Figure 3i and j). Subsequent Spearman rank correlation analysis confirmed a prominent correlation between serum miR-16 levels and melanoma progression from stage I to stage IV (Figure 2b). Tissue miR-16 expression levels also consistently showed prominent reduction in melanoma patients, which were significantly associated with AJCC stage (Figure 2c–e). These data confirmed that miR-16 levels, in both sera and tissues, had a high potential for determining melanoma progression as measured by disease stage.

**Significant correlation between serum miR-16 level and tissue Ki-67 expression**

Expression of the human Ki-67 protein is strictly associated with cell proliferation and is absent from resting cells. The fraction of Ki-67—positive tumor cells is often correlated with clinical stage so that Ki-67 expression is an important and precise indicator for progression and prognosis in cancers, including melanoma (Gimotty et al., 2005; Li et al., 2002; Nielsen et al., 2013; Scholzen and Gerdes, 2000). To further confirm the potential value of serum miR-16 level in determining the progression, we analyzed the correlation between serum miR-16 level and tissue Ki-67 expression. First, we performed immunohistochemistry to evaluate Ki-67 expression scores in tissue specimens from the 120 melanoma patients as well as 30 noncancerous nevus tissues. As expected, Ki-67 expression scores were significantly higher in melanoma tissues than in nevus tissues (Figure 3a–f). More importantly, Ki-67 expression scores increased as melanoma progressed from early to advanced stages, manifesting its credible role in determining melanoma progression (Figure 3f). We also found significant negative correlation between serum miR-16 level and Ki-67 expression scores (Figure 3g and h). Tissue miR-16 level and Ki-67 expression scores similarly were significantly correlated (Figure 3i and j).

**Serum miR-16 level as an independent prognosticator of melanoma patients**

The prognosis of melanoma patients varies substantially depending on progression status (Balch et al., 2009). Because we had validated the potential of serum miR-16 level in evaluating melanoma progression, we proposed that serum miR-16 level could also predict the prognosis of melanoma patients. Kaplan-Meier analysis was performed to compare the prognostic outcome of melanoma patients with different levels of serum miR-16. The median value 0.399 of all the 120 melanoma patients’ serum miR-16 levels was defined as the threshold. Serum miR-16 levels >0.399 were classified as high levels, whereas those ≤0.399 were regarded as low levels. Melanoma patients with low serum miR-16 levels had a significantly shorter survival compared with those with high serum miR-16 levels (log-rank test: $P < 0.0001$; Figure 4a). We then performed Cox proportional hazards regression analysis to assess the association between overall survival and serum miR-16 level in the presence of clinicopathologic characteristics. Univariate analysis showed that low serum miR-16 levels, advanced AJCC stage, increased tumor thickness, occurrence of ulceration, and presence of sun-exposed sites predicted poor prognosis of melanoma patients (all $P < 0.05$), whereas no such association was found between patient survival and age or sex (Table 1). Forward multivariate analysis including serum miR-16 level, age, sex, AJCC stage, tumor thickness, ulceration status, and tumor site revealed that serum miR-16 level was an independent prognosticator for melanoma patient survival (hazard ratio [HR] 2.49, confidence interval [CI] 1.10–5.63, $P = 0.028$). Likewise, AJCC stage (HR 3.41, CI 1.60–7.29, $P = 0.002$), tumor thickness (HR 2.84, CI 1.34–6.02, $P = 0.006$), ulceration status (HR 2.60, CI 1.26–5.40, $P = 0.010$), and tumor site (HR 2.40, CI 1.22–4.73, $P = 0.011$) were also valuable independent prognosticators (Table 1). Thereafter, we

---

**Figure 2. Association between melanoma American Joint Committee on Cancer (AJCC) stages and miR-16 levels both in sera and tissues.** (a) Serum miR-16 levels of melanoma patients at different AJCC stages. (b) Correlation between serum miR-16 levels and melanoma AJCC stages was tested by Spearman’s rank correlation analysis, with $r$ and $P$-values indicated. (c) Expression levels of miR-16 in 120 melanoma tissues and 120 matched normal nevus tissues. (d) Tissue miR-16 levels of melanoma patients at different AJCC stages. (e) Correlation between tissue miR-16 levels and melanoma AJCC stages was tested by Spearman’s rank correlation analysis, with $r$ and $P$-values indicated. Two-tailed Student’s $t$-test was used to analyze significant differences. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 

---

**Table 1.** Clinicopathologic characteristics, survival analysis, and univariate and multivariate analysis of melanoma patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Survival Analysis</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum miR-16 level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>Significant</td>
<td>HR 2.49, CI 1.10–5.63</td>
<td>HR 2.49, CI 1.60–7.29</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AJCC stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulceration status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sun-exposed site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---
performed stratified analysis to assess whether the indicative potential of serum miR-16 levels on patient survival was modified by some important clinicopathologic factors (see Supplementary Table S2). We found that patients with serum miR-16 level showed substantially increased risk of death in the presence or absence of concomitant clinicopathologic risk factors (e.g., older age, male, advanced AJCC stage, occurrence of ulceration, thicker tumor, and sun-exposed sites; Figure 4b). Taken together, these results demonstrated the high potential of serum miR-16 level to be a prognosticator for melanoma patients.

**Suppressive role of miR-16 in melanoma development**

Because miR-16 level was significantly dysregulated and shown to be associated with AJCC stage and Ki-67 expression in both sera and tissues, we proposed that miR-16 may be involved in melanoma development. Therefore, we investigated the effect of miR-16 levels on cell functions in melanoma cell lines. miR-16 expression plasmids were transfected into melanoma cell lines successfully (Figure 5a). We found that overexpressed miR-16 promoted melanoma cell apoptosis ($P < 0.01$ for WM793B, $P < 0.01$ for 451LU, $P < 0.01$ for A2058) and inhibited cell proliferation ($P < 0.001$ for WM793B, $P < 0.01$ for 451LU, $P < 0.01$ for A2058; Figure 5b and c). Overexpression of miR-16 also resulted in significant cell cycle arrest (Figure 5d). To further analyze the effect of miR-16 on melanoma growth in vivo, A2058 melanoma cells were transfected with miR-16 or miR-NC expression plasmids and then subcutaneously injected into 10 paired nude mice. The mice were observed for xenograft growth for 40 days. Overexpressed miR-16 led to a significant reduction in tumor volumes ($P < 0.01$; Figure 5e–g). Ki-67 staining revealed inhibited proliferation of melanoma xenograft in nude mice transfected with miR-16 overexpression plasmids compared with untransfected controls (Figure 5h). Subsequent bioinformatics analysis showed the candidate target genes of miR-16, including Bcl-2 and CCND1 (Figure 5i). Bcl-2 and CCND1 are important modulators of cell apoptosis and cell cycle progression. We have shown that there are target sequences of miR-16 in the 3′ UTR region of Bcl-2 and CCND1 mRNA. Overexpressed miR-16 induced down-regulation of Bcl-2 and CCND1 expressions in different melanoma cell lines (see Supplementary Figures S2a and c online). In addition, immunofluorescence and immunoblotting analysis showed significant down-regulation of BCL-2 and CCND1 expressions in melanoma xenograft transfected with miR-16 overexpressed plasmids (see Supplementary Figure S2d–f). Taken together, these results showed that miR-16 could suppress melanoma growth both in vitro and in vivo.

**DISCUSSION**

In this study, we identified serum miR-16 as a potential biomarker for evaluating progression and prognosis in melanoma patients. We found that serum miR-16 level was significantly reduced in melanoma patients compared with cancer-free controls. Furthermore, we found that...
serum miR-16 level was highly associated with tumor thickness, ulceration status, AJCC stage, and tissue Ki-67 expression. Moreover, we demonstrated that serum miR-16 level could independently predict melanoma patient survival.

Recent studies revealed that miRNAs not only could be detected in tissues but also could stably exist in human blood. The measurement of miRNAs in sera was established as an important approach for the blood-based detection of human cancers (Chen et al., 2008; Mitchell et al., 2008). For melanoma, several serum miRNAs have been validated for their value in patient diagnosis and prognosis. In 2010, Leidinger et al. (2010) reported that by using a subset of 16 significant dysregulated serum miRNAs, melanoma patients could be distinguished from healthy people with high accuracy, specificity, and sensitivity. Serum miR-221 was then found to be significantly increased in melanoma patients and of a great value in diagnosis and prognosis (Kanemaru et al.,...
Table 1. Univariate and multivariate analyses of clinical parameters and serum miR-16 expression levels in association with overall survival

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>miR-6 expression (&lt;0.399 vs. &gt;0.399)</td>
<td>2.46 (1.07–5.66)</td>
<td>0.035</td>
</tr>
<tr>
<td>Age at diagnosis (&lt;50 years vs. &gt;50 years)</td>
<td>1.00 (0.98–1.03)</td>
<td>0.895</td>
</tr>
<tr>
<td>Gender (female vs. male)</td>
<td>1.71 (0.83–3.50)</td>
<td>0.145</td>
</tr>
<tr>
<td>AJCC stage (I, II vs. III, IV)</td>
<td>2.90 (1.33–6.32)</td>
<td>0.008</td>
</tr>
<tr>
<td>Tumor thickness (≤1 mm vs. &gt;1 mm)</td>
<td>2.67 (1.25–5.71)</td>
<td>0.012</td>
</tr>
<tr>
<td>Ulceration (no vs. yes)</td>
<td>2.49 (1.20–5.18)</td>
<td>0.014</td>
</tr>
<tr>
<td>Site (sun exposed vs. sun protected)</td>
<td>2.56 (1.29–5.05)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Abbreviations: AJCC, American Joint Committee on Cancer; CI, confidence interval; HR, hazard ratio.

Multivariate Cox regression analyses were adjusted for all factors listed in Supplementary Table S2 online.

2011). Serum miR-206 later was also proved for its prognostic potential for melanoma patients (Tian et al., 2015). Recently, some serum miRNAs have also been reported to be associated with recurrence risk in primary melanoma patients (Fleming et al., 2015; Friedman et al., 2012). More importantly, it was reported that serum miR-16 level was significantly increased in melanoma patients compared with cancer-free controls, and there were no prominent changes of serum miR-16 level between AJCC stages (Stark et al., 2015), which was inconsistent with our results. The conflicting results may be due to the different sample sizes and human ethnicities included in the cohorts. Additional independent validation in other cohorts is needed to fully clarify the potential of serum miR-16 as a biomarker in melanoma.

Ki-67, the most widely used proliferation marker in pathology, is a nuclear antigen present in all active phases of cell cycle proliferation (G1, S, G2, and M) but absent in the quiescent phase (Chorny et al., 2003; Hazan et al., 2002; Henrique et al., 2000; Korabiowska et al., 2000; Li et al., 2000; Moretti et al., 2001; Rieger et al., 1993; Vogt et al., 1997). It has been reported that elevated Ki-67 expression is correlated with increased tumor thickness and the presence of tumor ulceration in melanoma (Ladstein et al., 2010). In addition, previous multivariate Cox regression analysis revealed that tissue Ki-67 expression could predict melanoma survival independently (Gimotty and Guerry, 2010; Gimotty et al., 2005). In this study, we established a previously unreported link between serum miRNA and the tissue molecular biomarker Ki-67 in melanoma.

Serum miRNAs are stable and easily accessed molecules in human blood. Through blood sampling, miRNA extraction, and sequential standardized PCR-based assays, serum miR-16 levels could be acquired immediately after diagnosis of melanoma patients with much convenience. In our study, multivariate analysis revealed that serum miR-16 was an independent prognostic factor after adjustment for tumor thickness, occurrence of ulceration, and AJCC stages, which were reported as valuable prognosticators in melanoma (Dickson and Gershwenwald, 2011). Noticeably, serum miR-16 could have more prognostic value in evaluating patient survival in specific subgroups, including patients of older age, advanced AJCC stage, increased tumor thickness, occurrence of ulceration, and sun-exposed tumor sites. Although serum miR-16 was the lowest ranking in multivariable analysis, it is still of essential suitability as a prognosticator for melanoma because of its lesser invasiveness, greater feasibility, and higher methodological stability compared with some applied prognosticators such as tumor thickness and Ki-67. However, the relevant threshold of serum miR-16 level for evaluating patient prognosis needs further investigation because of the limited sample size of the cohort in this study.

Accumulating evidence has revealed that miR-16 could function as a tumor suppressor in different kinds of cancers. For example, aberrant expression was observed in the tissue of ovarian cancer (Bhattacharya et al., 2009), myeloma (Sun et al., 2013), and leukemia (Cimmino et al., 2005). Prior findings of miR-16 serum and tissue expression in relation to tumor behavior and patient survival were summarized (see Supplementary Table S4 online). Therefore, we considered that miR-16 was cancer associated and may play important roles in melanoma, without exclusion of other dysregulated miRNAs we found in the microarray analysis. In this study, the level of miR-16 was found significantly decreased in both sera and tissues samples from melanoma patients. The hallmarks of cancer comprise six biological capabilities acquired during the multistep development of carcinogenesis: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis (Hanahan and Weinberg, 2011). In cancer, miR-16 could act as a pleiotropic modulator of these key biological capabilities. By targeting multiple cell cycle genes, miR-16 induced cell cycle arrest in HeLa, HepG2, and A549 cell lines and osteosarcoma (Cai et al., 2012; Liu et al., 2008). miR-16 could also promote apoptosis by regulating Bcl-2 in chronic lymphocytic leukemia (Cimmino et al., 2005). In addition, miR-16 has been reported to affect the angiogenesis of multiple myeloma by targeting vascular endothelial growth factor (Sun et al., 2013). Beyond this, migration and metastasis could be influenced by miR-16 in non-small cell lung cancer cells (Ke et al., 2013). Consistent with previous studies, we found that overexpressed miR-16 could markedly induce cell apoptosis, cell cycle arrest, and proliferation inhibition in melanoma cell lines. We also...
proved that miR-16 could suppress melanoma growth in a xenograft mouse model. These results suggested a critical role of miR-16 in melanoma development. Moreover, the results of in vivo study suggest that RNA mimics therapy by regulating miR-16 expression may be valuable in melanoma treatment.
This study has several limitations. First, because this study included a hospital-based case-control analysis of a relatively small sample size, an additional independent validation cohort from other institutions with larger sample sizes would be highly supportive. Second, we noticed that serum miR-16 levels in a small subgroup of melanoma patients were higher than those of the majority of cancer-free controls by quantitative real-time PCR validation analysis, indicating that for genetic heterogeneity, serum miR-16 could not represent all melanomas. Therefore, other potential miRNAs needed to be investigated, and combined miRNAs may be more valuable as biomarkers in melanoma. Finally, the cause of down-regulation of serum miR-16 in melanoma is not characterized. Because circulating miRNAs could originate from circulating blood cells, exosomes derived from tissues, and content of apoptotic/necrotic cells (Kosaka et al., 2010), down-regulation of serum miR-16 in melanoma patients could result from, for example, paracrine control of miR-16 from cutaneous microenvironment by melanoma. Elucidating the mechanism involved in the dysregulation of miRNA profiles in melanoma is difficult but worthy of attention in future studies.

MATERIALS AND METHODS
Further details are available in the Supplementary Materials and Methods (online).

Patients and specimens
This study included 120 melanoma patients: 30 patients with AJCC stage I (mean age 51.0 years, 18 females), 30 patients with AJCC stage II (mean age 50.0 years, 19 females), 30 patients with AJCC stage III (mean age 55.0 years, 15 females), and 30 patients with AJCC stage IV (mean age 54.2 years, 15 females) disease, as well as 120 matched cancer-free controls (mean age 48.0 years, 55 females) recruited from Xijing Hospital (Xi’an, China) between September 2004 and July 2013. All subjects were Chinese Han people with no previous systemic treatment. Blood samples were collected from the children of biopsy/surgery for melanoma tissues from patients or nevus tissues from cancer-free controls. Blood samples were then processed to obtain sera and stored at −80°C before extraction of miRNAs. All tissues were collected using standard procedures and also stored at −80°C. All specimens were collected with the approval of the Institutional Review Board of Fourth Military Medical University, Xi’an, China. Written informed consent was obtained from all patients and cancer-free controls, according to the principles of the Declaration of Helsinki.

CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENTS
The authors thank the doctors and the patients who participated in our study. This work was supported by the Program for New Century Excellent Talents at the University of China and the National Natural Science Foundation of China (81502863, 81572672).

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2015.12.041.

REFERENCES


Ladstein RG, Bachmann IM, Straume O, Akslen LA. Ki-67 expression is superior to mitotic count and novel proliferation markers PHF3, MCM4 and mitosin as a prognostic factor in thick cutaneous melanoma. BMC Cancer 2010;10:140.


