ZBTB7A Suppresses Melanoma Metastasis by Transcriptionally Repressing MCAM

Xue-Song Liu1, Matthew D. Genet1, Jenna E. Haines1, Elie K. Mehanna1, Shaowei Wu2, Hung-I Harry Chen3, Yidong Chen3, Abrar A. Qureshi4, Jiali Han2, Xiang Chen5, David E. Fisher6, Pier Paolo Pandolfi7, and Zhi-Min Yuan1

Abstract

The excessive metastatic propensity of melanoma makes it the most deadly form of skin cancer, yet the underlying mechanism of metastasis remains elusive. Here, mining of cancer genome data sets discovered a frequent loss of chromosome 19p13.3 and associated downregulation of the zinc finger transcription factor ZBTB7A in metastatic melanoma. Functional assessment of ZBTB7A-regulated genes identified MCAM, which encodes an adhesion protein key to melanoma metastasis. Using an integrated approach, it is demonstrated that ZBTB7A directly binds to the promoter and transcriptionally represses the expression of MCAM, establishing ZBTB7A as a bona fide transcriptional repressor of MCAM. Consistently, downregulation of ZBTB7A results in marked upregulation of MCAM and enhanced melanoma cell invasion and metastasis. An inverse correlation of ZBTB7A and MCAM expression in association with melanoma metastasis is further validated with data from analysis of human melanoma specimens.

Implications: Together, these results uncover a previously unrecognized role of ZBTB7A in negative regulation of melanoma metastasis and have important clinical implications. Mol Cancer Res; 13(8); 1206–17. ©2015 AACR.

Introduction

Melanoma is a malignant tumor of melanocytes and is classified by stage, based primarily on degree of local invasion or metastatic status of the malignant melanocytes. The prognosis of melanoma drops sharply as the stage progresses, and metastasis is primarily responsible for the poor prognosis of cutaneous melanoma (1). Melanoma is thought to progress through multiple steps before distant metastatic spread to organs such as the liver and lung (2). The molecular changes associated with the transition of melanoma cells through to the most advanced phases are not fully understood.

In order to metastasize from primary sites, tumor cells must attach and invade through endothelial cells, implicating the critical roles cell adhesion molecules play in the metastatic progression of melanoma (3). One such cell adhesion molecule, melanoma cell adhesion molecule (MCAM), is widely known to play a pivotal role in the metastatic progression of melanoma (4). Reported as a valuable prognostic marker for melanoma, MCAM is highly expressed in advanced stage melanoma but not in benign melanocytes (5–7). Furthermore, forced expression of MCAM in nonmetastatic melanoma cells strongly enhances metastatic potential, while MCAM antibody treatment significantly suppresses metastatic progression of human melanoma in vivo (8, 9). However, how MCAM expression is upregulated during human melanoma progression is not well understood.

Genetic alterations, such as recurrent chromosomal alterations, can be primary causes for many human cancers (10). Chromosomal focal amplifications or deletions often produce copy number variation (CNV) of genes, which may contribute to tumor progression. These chromosomal alterations can lead to deregulation of gene structure, function, and expression that functionally contribute to the pathogenesis of cancer (10). A recent study by The Cancer Genome Atlas (TCGA) Pan-Cancer Analysis Working Group performed an integrative analysis of somatic copy number alterations across 12 tumor types and provided a public resource of highly curated data and information (11). We performed data mining of the TCGA database and identified 19p13.3 as a region of frequent chromosomal deletion in metastatic melanoma. To investigate the biologic significance of this chromosomal loss to melanoma, we analyzed genes localized within this region and discovered that ZBTB7A, a transcriptional repressor with Zinc-finger and BTB/POZ domains, was significantly downregulated during progression from normal skin and benign nevus to late-stage melanoma. We demonstrate that ZBTB7A suppresses melanoma metastasis by transcriptionally repressing the critical melanoma progression molecule MCAM in vitro and in vivo, implicating ZBTB7A as a novel tumor suppressor against melanoma metastasis.
Materials and Methods

Promoter mutation

ZBTB7A-binding sites in human MCAM promoter region were mutated with Q5 Site-Directed Mutagenesis Kit (New England Biolabs). Mutation primers were designed using NEBaseChanger software provided by the Q5 Mutagenesis Kit. Mutation PCR, Kinase-Ligase-DpnI (KLD) enzyme treatment and transformation were performed according the manufacturer’s instructions. All the mutations have been confirmed via sequencing.

Luciferase assay

The 293 or SK-MEL-28 cells were cultured in a 48-well plate, and transfection was started when cell density was approximately 50% confluence. Of note, 20 ng of pGL3-MCAM vector, 10 ng of pRL-CMV Renilla luciferase reporter and pcDNA3.1 control, or increasing dose (5–30 ng) of pcDNA3.1-ZBTB7A expression plasmid were cotransfected into 293 cells. After 36 hours, the luciferase activity was measured with the dual-luciferase reporter assay system (Promega) according to the manufacturer's instructions.

Tissue Microarray analysis

The Tissue Microarray ME2082 and ME1003 used in the study was purchased from US Biomax, stained and analyzed at the Pathology and Molecular Cytology Core facilities. ME2082 contains 128 cases of primary malignant melanoma, 64 metastatic malignant melanoma, and 16 cases of adjacent normal tissue or normal tissue from autopsy. ME1003 contains 56 cases of malignant melanoma, 20 cases of metastatic malignant melanoma, and 24 cases of benign nevi.

Lentiviral shRNA-mediated knockdown

293T cells were seeded in 10-cm tissue culture plates, and plasmid transfection was performed when the cell reach 60% confluence. Before transfection, 293T cells culture medium was changed to antibiotics free DMEM medium with 10% FBS. Of note, 10 μg of shZBTB7A plasmid (Sigma, TRCN0000137332), shMCAM plasmid (Qiagen, KH00651N) or control nontargeting shRNA plasmid, 7.5 μg of psPAX2 packaging plasmid, and 2.5 μg of pMD2.G envelope plasmid were cotransfected using 20 μL Lipofectamine 2000 Transfection Reagent (Invitrogen). The next day after transfection, the transfection medium was changed to fresh complete culture medium. The virus supernatant was collected every 12 hours, filtered through a 0.45-μm filter, and frozen in –80°C until further usage. The virus titer was determined by serial dilution assay using 2T3 cells. For melanoma cell lentiviral transfection, 5 × 10⁵ cells were seeded in 10-cm dish, incubated with virus at MOI = 1 for 12 hours in the presence of 8 μg/mL polybrene (Sigma). Seventy-two hours later, the cells were selected with 1 μg/mL of puromycin (for shZBTB7A) for 3 days or 500 μg/mL of G418 (for shMCAM) for 1 week. The polyclonal cells after selection are used for downstream experiments.

Melanoma cell nude mice lung seeding assay

All animal procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the Harvard School of Public Health (Boston, MA). A total of 1 × 10⁶ melanocytes of different genotype were injected through tail vein into nude mice (nu/nu, female, 6–8 weeks old, Charles River Laboratories). Sixty days later, nude mice lung were dissected, fixed with 4% formalin, and embedded in paraffin blocks. Sections of lung were stained with hematoxylin and eosin. Degree of lung seeding was determined by histopathological scoring of infiltrating melanoma cells percentage among normal lung cells.

Statistical analysis

Log-rank tests were used for Kaplan–Meier survival analysis. The Student t test was used for the comparisons of the means. Correlations of gene expression were determined using Pearson coefficient. All statistical tests were two-sided. P values of less than 0.05 were considered statistically significant.

Results

19p13.3 is frequently deleted in human melanoma, and ZBTB7A is the top down-regulated gene within this region

Genome-wide SNP array analysis has uncovered several recurrent chromosomal alterations (12). We sought to understand the genetics of human melanoma metastasis by mining several human genomic databases and discovered 19p13.3 as one of the frequently deleted chromosomal regions, which is downregulated in approximately 30% (47 of total 158) of melanoma cases (Fig. 1A). Of note, chromosomal 19p13.3 is gradually lost along the progression of melanoma, as reflected by a significant drop from primary in situ melanoma to metastatic melanoma (Fig. 1B). Consistent with the close association of metastasis with mortality, data derived from TCGA Skin Cutaneous Melanoma datasets reveal that decreased chromosome 19p13.3 copy number correlates with poor prognoses in melanoma patients (Fig. 1C).

To explore the critical genetic events associated with 19p13.3 loss in melanoma metastasis, we examined the expression of genes within this region relative to disease progression. Among the genes found within the 19p13.3 region (Supplementary Fig. S1), only a few were found to be significantly altered during disease progression according to two different datasets (refs. 13, 14; Supplementary Fig. S2). ZBTB7A was one of the top down-regulated genes in both datasets (Supplementary Fig. S2), and importantly, its expression significantly decreases progressively from normal skin to nevus to melanoma (Fig. 1D). Known cancer genes as defined by The Sanger Institute’s Cancer Gene Census in the 19p13.3 region include STK11, GNA11, TCF3, FSTL3, and SIRT6 (15), none of which exhibited the same correlation as ZBTB7A, and no discernable pattern of regulation was detected during disease progression (Fig. 1D). In addition, no significant somatic mutations have been found in any of these known cancer genes in the TCGA skin cutaneous melanoma (SKCM) somatic mutation database (16), diminishing the likelihood that any of these genes functionally link to 19p13.3 loss during melanoma metastasis. The data together suggest ZBTB7A as a possible cancer gene candidate in 19p13.3 loss-associated melanoma.

ZBTB7A is downregulated in human melanoma metastases

ZBTB7A [also known as POKEMON (17), FBI (18), LRP (19) and OCZF (20)] is a member of the POZ/BTB and Kruppel (POK) family of transcription factors. POK family transcription factors can bind DNA through Zinc finger domain and repress transcription by recruiting corepressor complexes through the BTB (for BR-C, ttk, and bab) domain (21). POK transcription factors have been recognized to be critical developmental regulators and have been directly implicated in human cancers (22–24). Analysis of CNV values indicates that ZBTB7A copy number was significantly
Figure 1. 19p13.3 deletion associated ZBTB7A downregulation contributes to melanoma metastasis and poor prognosis. A, list of significant chromosomal focal deletions in human melanoma based on the TCGA SKCM SNP array dataset. GISTIC q-values (x-axis) are plotted across different chromosome (y-axis). B, 19p13.3 CNV status in primary and metastatic melanoma based on TCGA SNP array database, error bars represent mean ± SEM. C, Kaplan–Meier overall survival curve of patients with and without 19p13.3 deletion based on TCGA melanoma dataset. D, expression of 19p13.3 cancer genes STK11, TCF3, FSTL3, GNA11, SIRT6, and ZBTB7A during the progression of human melanoma, error bars represent mean ± SEM.
Reduced in metastatic melanoma when compared with primary melanoma (Fig. 2A). In line with these CNV data, the mRNA expression levels of \(ZBTB7A\) also declined significantly upon disease progression (Fig. 2B). To corroborate these data derived from the human melanoma datasets, we analyzed a panel of 12 human melanoma cell lines. The result confirmed the correlation between the CNV and mRNA of \(ZBTB7A\) (Fig. 2D–F), implicating CNV as a recurrent alteration at the \(ZBTB7A\) locus that contributes to downregulation of its expression in human melanoma. Significantly, lower expression of \(ZBTB7A\) mRNA also correlates with poor prognosis (Fig. 2G).

An inverse correlation between \(ZBTB7A\) and MCAM expression in human melanoma samples and cell lines

Given that \(ZBTB7A\) is a transcriptional repressor, we began to explore its role in melanoma development by identifying its potential target genes. Microarray analysis was performed to uncover differentially expressed genes between \(ZBTB7A\)-proficient and deficient cells. Gene set enrichment analysis revealed cell adhesion molecules as a top gene signature enriched in differentially expressed genes (Supplementary Fig. S3A). Significantly, MCAM (Melanoma Cell Adhesion Molecule), a critical regulator of melanoma metastasis and progression, was found to be the top significantly upregulated cell adhesion molecule in \(ZBTB7A\)-deficient cells (Supplementary Figs. S3B and S4). MCAM, also known as MUC18, CD146, Mel-CAM, A32 antigen, and S-Endo-1 Antigen, is a membrane glycoprotein, belonging to the immunoglobulin superfamily. It was previously identified as a marker of melanoma progression and metastasis (25).

The highly elevated MCAM expression in \(ZBTB7A\)-deficient cells prompted us to examine its expression levels in human clinical melanocytic lesions. In sharp contrast with the association of decreased \(ZBTB7A\) expression with melanoma metastasis, MCAM expression was seen to progressively increase from nevus...
Figure 3. ZBTB7A expression negatively correlates with MCAM expression in both human melanoma patient samples and melanoma cell lines. A, the levels of ZBTB7A mRNA and MCAM mRNA in different stages of human melanoma were analyzed based on the values derived from Kabbarah dataset. Error bars represent mean ± SEM. B, the correlation between ZBTB7A and MCAM mRNA expression derived from two different human melanoma datasets was shown. C, the mRNA levels of 12 human melanoma cell lines were assessed by real-time PCR, 18s served as internal control for the analysis. Average values of three independent experiments are shown as mean ± SEM. D and E, the correlation of ZBTB7A mRNA with MCAM mRNA in 12 human melanoma cell lines was analyzed. ZBTB7A low expression cell lines (A375, SK-Mel-2, UACC62, and UACC257) express an average 12-fold more MCAM than ZBTB7A high expression cell lines (1205Lu, Lox-IM VI, MeWo, and WM155). F, ZBTB7A and MCAM protein expression detected by Western blot analysis in 12 different human melanoma cell lines, β-actin served as loading control.
ZBTB7A Suppresses Melanoma Metastasis through MCAM

ZBTB7A transcriptionally represses MCAM expression in vitro and in vivo

To test the hypothesis that ZBTB7A transcriptionally represses MCAM expression, we knocked down the expression of ZBTB7A with three independent siRNA sequences in multiple melanoma cell lines M14, SK-Mel-5, 1205Lu, and UACC257. ZBTB7A knockdown was associated with a robust induction of the expression of MCAM at both protein and mRNA levels in all four melanoma cell lines (Fig. 4A and B), supporting a ZBTB7A-dependent regulation of MCAM expression. To complement the knockdown approach, we overexpressed ZBTB7A in two melanoma cell lines SK-Mel-28 and UACC62 that express a relatively low endogenous level of ZBTB7A. The expression of MCAM protein and mRNA was strongly suppressed upon introduction of exogenous ZBTB7A (Fig. 4C and D). These results are consistent with the hypothesis that ZBTB7A suppresses the transcription of MCAM. To further validate the ZBTB7A-dependent regulation of MCAM expression, we generated ZBTB7A stable knockdown and control M14 melanoma cells using shRNA and implanted the cells into nude mice. IHC staining of tumor sections derived from implanted cells revealed that knockdown of ZBTB7A was associated with a significant decrease in MCAM expression (Fig. 6A, left). Overexpression of ZBTB7A in UACC62 cells resulted in considerable increase in M14 cell adhesion to HUVEC cells (Fig. 6A, left). This increased adhesion was completely abolished by anti-MCAM antibody (relative to control antibody), demonstrating that the effect is mediated by MCAM repressor (Fig. 6A, left). Overexpression of ZBTB7A in UACC62 cells, on the other hand, was associated with a considerable reduction of cell adhesion to HUVEC cells (Fig. 6A, right). These data together indicate that by controlling MCAM expression, ZBTB7A plays a critical role in regulation of melanoma cell adhesion to HUVEC cells. A Matrigel-based invasion assay was also carried out to assess the role for ZBTB7A-dependent regulation of MCAM expression. The results indicate that ZBTB7A knockdown stimulated cell invasion and this effect of ZBTB7A-deficiency was mitigated by the use of the anti-MCAM antibody. Conversely, ZBTB7A overexpression suppressed cell invasion (Fig. 6B). Finally, we tested the role of
A

<table>
<thead>
<tr>
<th></th>
<th>M14</th>
<th>sk-mel-5</th>
<th>1205Lu</th>
<th>UACC257</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiCon</td>
<td>SiZBTB7A</td>
<td>SiZBTB7A</td>
<td>SiZBTB7A</td>
<td>SiZBTB7A</td>
</tr>
<tr>
<td>1#</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2#</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3#</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SiCon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B

**ZBTB7A**

![Bar graph showing relative mRNA expression for ZBTB7A](image)

**MCAM**

![Bar graph showing relative mRNA expression for MCAM](image)

C

![Western blot showing MCAM and ZBTB7A](image)

D

**MCAM**

![Western blot showing MCAM and ZBTB7A](image)

**ZBTB7A**

![Western blot showing MCAM and ZBTB7A](image)

E

**ZBTB7A**

![Immunofluorescence images showing ZBTB7A](image)

**MCAM**

![Immunofluorescence images showing MCAM](image)
ZBTB7A in melanoma metastasis using a nude mouse lung seeding assay. When compared with control shRNA-expressing M14 cells, the shZBTB7A-expressing melanoma cells developed much more lung metastasis, which was completely diminished by MCAM knockdown (Fig. 6C). Quantitative analysis of the lung tumor nodules revealed an approximate 5-fold increase in lung metastasis in ZBTB7A-deficient melanoma cells and remarkably, this increase was nearly completely eliminated when MCAM expression was knocked down (Fig. 6D). Together, our data demonstrate a critical role of ZBTB7A in control of melanoma

Figure 4.
ZBTB7A regulates the expression of MCAM in vitro and in vivo. A, the indicated melanoma cell lines were transfected with control (SiCon) or three independent ZBTB7A targeting siRNAs. The cells were harvested 48 hours after transfection and analyzed by immunoblot analysis with MCAM and ZBTB7A-specific antibodies. β-actin served as loading control. B, the mRNA levels of ZBTB7A and MCAM were assayed by real-time PCR in M14 cells after ZBTB7A knockdown with three different siRNA. Data shown represent mean ± SD of three experiments. C, retrovirus mediated ZBTB7A overexpression in melanoma cell lines SK-MEL-28 and UACC62. Immunoblot analysis was performed with MCAM and ZBTB7A-specific antibodies. β-actin served as internal control for loading. D, the mRNA levels of ZBTB7A and MCAM were assayed by real-time PCR in SK-MEL-28 and UACC62 cells after retrovirus mediated ZBTB7A overexpression. Data shown are mean ± SD of three experiments. E, M14 cells stably expressing lentiviral shZBTB7A and shcontrol vector were subcutaneous injected into nude mice. The developed tumors were dissected, fixed, and stained with ZBTB7A or MCAM specific antibody. Bar, 50 μm.
metastasis and this transcription repressor does so via regulation of the expression of MCAM.

To further confirm the association of ZBTB7A and human melanoma, we examined the expression of ZBTB7A protein in a cohort of human melanoma samples by IHC staining. The results indicate a gradual loss of ZBTB7A protein expression during progression of melanoma. The expression of ZBTB7A is readily detectable in normal skin and benign nevi, considerably reduced in melanoma in situ, and almost undetectable in metastatic melanoma (Fig. 7A), displaying an inverse correlation with ZBTB7A expression and melanoma progression (Fig. 7B). The expression of MCAM, on the other hand, exhibited a positive correlation with disease progression. The MCAM level was barely detectable in normal skin but increased progressively from nevus to primary melanoma to metastatic melanoma (Fig. 7C–E). The inverse correlation of ZBTB7A and MCAM expression in association with melanoma progression is in agreement with that uncovered from the TCGA database.

Discussion

The cancer genomic projects have produced rich data serving as valuable resources for cancer research (12). We conducted data mining of the TCGA database and identified 19p13.3 as a recurrently deleted region in melanoma. We discovered decreased copy number of ZBTB7A, a gene localized within 19p13.3, as a major genetic event with a measurable biologic significance. Consistent with its function as a transcription repressor, we identified melanoma cell adhesion molecule (MCAM) as a target gene under the control of ZBTB7A. Indeed, in vitro and in vivo evidence showed...
An inverse correlation between ZBTB7A and MCAM in human melanoma. A, detection of ZBTB7A protein by IHC in tissue microarrays of human normal skin, benign nevus, primary and metastatic melanoma samples. Bar, 50 μm. B, statistical analysis of ZBTB7A expression levels in normal skin, benign nevus, primary and metastatic melanoma tissues. ZBTB7A is expressed in 100% (16 of 16) normal skin, 83% (20 of 24) benign nevus, 41% (76 of 184) primary melanoma, and 12% (10 of 84) metastatic melanoma. C, detection of MCAM protein by IHC in tissue microarrays of human normal skin, benign nevus, primary and metastatic melanoma samples. Bar, 50 μm. D, statistical analysis of MCAM expression levels in normal skin, benign nevus, primary and metastatic melanoma samples. MCAM is expressed in 0% (0 of 16) normal skin, 8% (2 of 24) benign nevus, 38% (70 of 184) primary melanoma, and 76% (64 of 84) metastatic melanoma. E, an inverse-correlation between ZBTB7A and MCAM expression in tissue microarray samples. F, model for the function of ZBTB7A in the regulation of melanoma progression and metastasis.
that downregulation of ZBTB7A resulted in robust induction of MCAM, promoting melanoma metastasis. ZBTB7A is a POK family transcription repressor that is best known for its proto-oncogenic role through repressing the expression of tumor suppressor ARF (17). The finding that 19p13.3 loci containing ZBTB7A is frequently lost in human melanoma seems to be inconsistent with an oncogenic role of ZBTB7A in skin tumorigenesis. Analysis of several melanoma genomics databases revealed that the expression levels of ZBTB7A decrease progressively among normal skin, nevi, primary melanoma, and metastatic melanoma, largely due to a reduction of copy number caused by 19p13.3 loss. Significantly, among the genes localized within 19p13.3, ZBTB7A was found to be the gene whose expression exhibits a reverse correlation with melanoma progression, arguing against an oncogenic role of ZBTB7A in human melanoma. Consistent with this notion is the finding that, similar to 19p13.3 loss, reduced expression of ZBTB7A is closely associated with poor prognosis of melanoma patients. ZBTB7A is a known transcriptional repressor (17). Gene set enrichment analysis of differentially expressed genes between ZBTB7A-proficient and deficient cells revealed cell adhesion molecules as the most enriched gene signature. MCAM, a critical regulator of melanoma metastasis and progression, was among the significantly upregulated genes in ZBTB7A-deficient cells. Using a combination of loss- and gain-of-function approaches, we confirmed ZBTB7A-dependent control of MCAM expression. The multiple GC-rich sequences matching consensus-binding sites for ZBTB7A were found in the MCAM promoter, and provide a structural basis for ZBTB7A-mediated regulation. Indeed, data obtained from experiments using complementary approaches, including ChIP and site-directly mutagenesis demonstrated direct binding of ZBTB7A to the MCAM promoter, thereby establishing ZBTB7A as a bona fide transcriptional repressor of MCAM. Interestingly, the MCAM genomic locus is rarely amplified (Supplementary Fig. S5B), supporting transcriptional regulation as the major mechanism of MCAM control. The importance of ZBTB7A-mediated suppression of MCAM expression in human melanoma is further supported by data derived from our analysis of a cohort of human melanoma patient samples, which showed a clear inverse correlation of ZBTB7A and MCAM expression in association with melanoma progression. Recently, we identified a tumor-suppressive function of ZBTB7A by repressing the transcription of glycolysis genes (29). In human colon cancers, we observed significantly reverse correlation between ZBTB7A and glycolysis genes (29), whereas in melanoma, ZBTB7A do not show significant reverse correlation with glycolysis genes, and similarly we do not observe the reverse correlation between ZBTB7A and MCAM in human colon cancers. Most importantly, depletion of MCAM nearly completely rescued the melanoma metastasis phenotype of ZBTB7A-deficient melanoma cells in vitro and in vivo. Our study indicates cancer type specific mechanisms for ZBTB7A in cancer suppression, and MCAM plays a major function in ZBTB7A regulated melanoma metastasis.

MCAM has been identified as a major contributor to melanoma progression and metastasis. This cell adhesion protein is highly expressed in a wide range of advanced and metastatic melanoma, but is rarely expressed in benign nevus cells or normal melanocytes (26), implicating that the upregulation of MCAM is late event during melanoma progression. We also analyzed the genetic information of the cell lines used in this study for the association. Among the melanoma lines, MeWo harbors wild-type BRAF, NRAS, and PTEN; A375, Lex-IM VI, M14, SK-MEL-2, SK-MEL-5, and UACC62 have mutations in BRAF or NRAS, but wild-type for PTEN; SK-MEL-28 and UACC62 have mutations in BRAF or NRAS, but homozygous or heterozygous loss of PTEN, representing more advanced stage of melanoma. Interestingly, MeWo cells express a relatively high level of ZBTB7A, and a low level of MCAM. SK-MEL-28 and UACC62, on the other hand, express a low level of ZBTB7A that correlates with a high MCAM level. The correlation derived from melanoma cell lines is consistent with the notion that ZBTB7A downregulation and MCAM upregulation underscore a late genetic event in melanoma progression. Chromosome 19p13.3 loss and its associated reduction of ZBTB7A expression are likely the result of genomic instability. The resulting upregulation of MCAM may represent an important consequence to these genetic alterations during melanoma progression. MCAM expression correlates with tumor metastatic potential, and has been used as a prognostic marker for melanoma (5–7). In agreement with published work (30), we showed that ZBTB7A overexpression repressed the expression of MCAM resulting in diminished ability of melanoma cells to adhere to endothelial cells and to invade. ZBTB7A underexpression in melanoma cells was associated with enhanced cell adhesion and invasion, both of which were completely blocked by the use of anti-MCAM antibody. The importance of ZBTB7A-dependent transcriptional repression of MCAM in the suppression of melanoma metastasis was additionally corroborated by animal studies. Our data collectively suggest that reduced expression of ZBTB7A due to chromosome 19p13.3 loss is an important genetic event contributing to the progression of melanoma.

In summary, our study has uncovered a novel and common genetic event associated with human melanoma metastasis, that is, a frequent loss of chromosome 19p13.3. Biologic significance of this chromosome loss is highlighted by decreased expression of ZBTB7A, leading to enhanced melanoma progression and metastasis due to compromised repression of MCAM expression (Fig. 7F). The ZBTB7A-mediated transcriptional control of MCAM expression not only represents a previously unrecognized mechanism of regulation, but also carries therapeutic implications.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: X.-S. Liu, M.D. Genet, P.P. Pandolfi, Z.M. Yuan

Development of methodology: X.-S. Liu, M.D. Genet, S. Wu

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): X.-S. Liu, E.K. Mehanna, S. Wu, J. Han

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): X.-S. Liu, J.E. Haines, E.K. Mehanna, S. Wu, H.-J. Chen, Y. Chen, A.A. Qureshi, J. Han, D.E. Fisher

Writing, review, and/or revision of the manuscript: X.-S. Liu, J.E. Haines, S. Wu, A.A. Qureshi, D.E. Fisher, Z.M. Yuan

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): X. Chen

Acknowledgments

The authors thank the current and former members of the Yuan lab for experimental support, advice, and helpful discussions, The Nikon Imaging Center at Harvard Medical School for help in H&E and IHC image acquisition.
Grant Support
This work was supported in part by the Morningside Foundation and grants from the NIH/NCI (R01CA085679, RO1CA167814, and RO1CA125144).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received April 7, 2015; accepted April 22, 2015; published OnlineFirst May 20, 2015.

References

31. ZBTB7A Suppresses Melanoma Metastasis through MCAM

www.aacnjournals.org Mol Cancer Res; 13(8) August 2015 1217
ZBTB7A Suppresses Melanoma Metastasis by Transcriptionally Repressing MCAM

Xue-Song Liu, Matthew D. Genet, Jenna E. Haines, et al.