TWIST1 Is an ERK1/2 Effector That Promotes Invasion and Regulates MMP-1 Expression in Human Melanoma Cells

Michele B. Weiss¹, Ethan V. Abel¹,⁴, Melanie M. Mayberry¹, Kevin J. Basile¹,⁴, Adam C. Berger², and Andrew E. Aplin¹,³

Abstract

Tumor cells often use developmental processes to progress toward advanced disease. The E-box transcription factor TWIST1 is essential to epithelial–mesenchymal transition (EMT) and cell migration in the developing neural crest. In melanoma, which derives from the neural crest cell lineage, enhanced TWIST1 expression has been linked to worse clinical prognosis. However, mechanisms underlying TWIST1 expression and whether aberrant TWIST1 levels promote steps in melanoma progression remain unknown. Here, we report that elevated TWIST1 mRNA/protein expression is dependent on extracellular signal-regulated kinase (ERK)1/2 signaling, which is hyperactive in the majority of melanomas. We show that TWIST1 protein levels are especially high in melanoma cell lines generated from invasive, premetastatic stage tumors. Furthermore, TWIST1 expression is required and sufficient to promote invasion through Matrigel and spheroid outgrowth in three-dimensional dermal-mimetic conditions. Alterations to spheroid outgrowth were not as a result of altered cell death, cell-cycle profile, or paradigm EMT protein changes. Importantly, we identify matrix metalloproteinase-1 (MMP-1) as a novel downstream target of TWIST1. We have determined that TWIST1 acts, in a dose-dependent manner, as a mediator between hyperactive ERK1/2 signaling and regulation of MMP-1 transcription. Together, these studies mechanistically show a previously unrecognized interplay between ERK1/2, TWIST1, and MMP-1 that is likely significant in the progression of melanoma toward metastasis. Cancer Res; 72(24): 6382–92. ©2012 AACR.

Introduction

The process of invasion allows tumor cells to traverse the boundaries of the basement membrane and move through the ECM to intravasate and ultimately colonize distant sites (1). Often, tumor cells will use core developmental processes to progress toward advanced or metastatic disease. One such process is the epithelial–mesenchymal transition (EMT). EMT prompts morphologic and cytoskeletal changes in precursor cells that allow for the migratory reorganization of embryonic germ layers and is required for mesoderm formation during gastrulation (2). EMT or EMT-like processes are thought to be a critical step in the metastatic cascade by regulating motility and/or invasion, resistance to anoikis, and stem cell-like properties (3).

One important EMT regulator during development is the basic helix–loop–helix transcription factor, TWIST1 (2–5). It was originally discovered as a gene crucial for proper gastrulation and mesoderm formation in Drosophila (4). In mammals, TWIST1 expression during a precise time frame in embryogenesis allows for the migration and differentiation of several mesodermal and neural crest cell lineages (5, 6). Many of the phenotypes attributed to TWIST1 occur as a result of its binding to E-box consensus sites in gene promoters, ultimately leading to transcriptional activation or repression (4, 7).

TWIST1 is overexpressed in many primary tumors including colon, breast, prostate, and gastric carcinomas (8–11). In agreement with its role in embryonic cell migration, TWIST1 overexpression has been linked to increased tumor cell migration, invasion, and metastasis (7, 11–13). These actions of TWIST1 have been correlated with changes in classical EMT targets such as E-cadherin and N-cadherin (7, 11, 12); however, the extent to which TWIST1 regulates non-EMT targets is not fully understood.

Recently, TWIST1 was found to be highly upregulated in the vast majority of melanoma tumors and cell lines, and was correlated to worse patient survival (8, 14). Melanoma is an aggressive skin cancer that arises from neural crest–derived melanocytes (15, 16). Invasion plays a critical role in melanoma progression. If cells are mainly confined to expansion within the epidermis (radial growth phase, RGP), melanoma is easily cured through surgical intervention (15, 16). If undiagnosed...
and properties of invasion begin to emerge, cells escape the basement membrane and expand through the deeper dermal layers. This conversion to vertical growth phase (VGP) is the direct precursor to metastasis (15). The depth of melanoma invasion and tumor thickness are used as predictors of poor clinical prognosis (17, 18); however, the mechanisms underlying melanoma invasion from the epidermis into the dermis remain poorly characterized. Upregulation of the RAS-RAF-MAP–ERK kinase (MEK)-extracellular signal-regulated kinase (ERK)1/2 signaling pathway may be critically important in this process. Hyperactivation of this pathway is common in multiple cancer types but especially in melanoma, where mutations in N-RAS (15%–20%) or B-RAF (40%–60%) are prevalent (15, 16, 19). In addition, mutant B-RAF, especially B-RAFV600E, is required for enhanced growth and invasion of melanoma cells (20). Many of the factors influencing increased melanoma invasion downstream of RAS-RAF-MEK-ERK1/2 are unknown.

Results

TWIST1 is upregulated in melanoma cell lines, particularly in VGP, downstream of oncogenic B-RAF and is positively regulated by active ERK1/2 signaling

We explored the TWIST1 expression profile across an extensive panel of melanoma cell lines representing various tumor stages and genotypes. Our results show that TWIST1 protein is upregulated in all melanoma cell lines tested compared with neonatal human epidermal melanocytes (NHEM; Fig. 1A). Furthermore, TWIST1 protein is especially high in invasive VGP tumors compared with RGP and metastatic tumors of similar genotype, which may denote a selective requirement of TWIST1 in early steps of metastasis.

Interestingly, protein expression of TWIST1 in mutant B-RAF melanoma cell lines is elevated compared with similar stage wild-type B-RAF cell lines and correlates relatively well with the levels of phosphorylated ERK1/2 (Fig. 1A). Furthermore, we found that siRNA-mediated depletion of either mutant B-RAF or mutant and wild-type B-RAF greatly reduced TWIST1 protein levels in WM793 (B-RAFV600E) and WM115 (B-RAFV600E) cells (Fig. 1B). To eliminate the possibility of off-target effects, a rescue experiment was conducted in which inducible B-RAF knockdown cells were engineered to express a GFP control or to reexpress a short hairpin RNA (shRNA)-resistant form of B-RAFV600E. Upon rescue of B-RAFV600E expression, with concomitant reestablishment of phosphorylated ERK1/2, TWIST1 protein levels were restored (Fig. 1C). This was further confirmed by the inhibition of RAF signaling by PLX4720 (a nonclinical analog of PLX4032/vemurafenib), and by the inhibition of active ERK1/2 signaling by the MEK inhibitor, U0126 (Fig. 1D).

To extend our studies further, we used 2 independent metastatic melanoma samples surgically resected from patients. Both tumors were genotyped: tumor #1 harbors wild-type N-RAS and wild-type B-RAF, whereas tumor #2 harbors wild-type N-RAS and B-RAFV600E (data not shown). After short-term culture and transient MEK inhibition, both tumor samples evidenced downregulation of TWIST1 protein concurrent with reduction in phosphorylated ERK1/2 (Fig. 1E).

Individual knockdown of ERK1 and ERK2 in mutant B-RAF cell lines revealed that both isoforms contribute to regulation of TWIST1 (Fig. 1F). The amount of TWIST1 ablation correlated closely with the total amount of phosphorylated ERK1/2 remaining after knockdown. Despite links with the ERK1/2 pathway, inhibition of ribosomal S6 kinase (RSK; ref. 22) or STAT3 (23) did not result in TWIST1 protein changes (Supplementary Fig. S1A and S1B). In addition, AKT does not seem to contribute to TWIST1 regulation (Supplementary Fig. S1C; ref. 13). Thus, the molecule(s) that acts as a bridge between ERK1/2 and TWIST1 still remains elusive and requires future analysis.

TWIST1 regulation by active ERK1/2 signaling occurs at the transcript level, as TWIST1 mRNA is downregulated upon treatment with PLX4720 or PLX4032 and is further confirmed by the use of U0126 (*, \(P < 0.05\), **, \(P < 0.01\); Fig. 1G). In addition, while TWIST1 stability may be regulated posttranslationally in other cell types (24), the combination of cycloheximide and U0126 did not hasten TWIST1 protein degradation in mutant B-RAF melanoma cells (Supplementary Fig. S1D). Therefore,
we conclude that TWIST1 mRNA is positively regulated downstream of mutant B-RAF via activation of ERK1/2 signaling. TWIST1 is required and sufficient for melanoma cell invasion through Matrigel. The role of TWIST1 expression in the biology of advanced melanoma cells is currently unknown. Oncogenic RAF-MEK-ERK signaling has been shown to be critical for the invasive capabilities of melanoma cells (20, 25, 26). In addition, studies in breast cancer have implicated TWIST1 in the early invasive steps of the metastatic cascade (7). To assess the role of the ERK target, TWIST1, in the ability of melanoma cells to invade in a Matrigel transwell assay, we used the VGP cell lines WM793 and WM115. Reduced expression of TWIST1 protein via 3 independent shRNAs in WM793 and WM115 cells significantly reduced the invasive capacity of those cells by 40% to 50% (*, P < 0.05; **, P < 0.01). Conversely, we overexpressed TWIST1 in poorly invasive, RGP cell lines that displayed low endogenous TWIST1 expression. TWIST1 overexpression in Sbcl2 and WM3211 cells significantly increased the ability of melanoma cells to invade through Matrigel.
cells to invade through Matrigel by approximately 40% and 30%, respectively (\*, P < 0.01; Fig. 2C and D).

High TWIST1 expression promotes melanoma spheroid outgrowth

Many melanoma cell lines have the ability to aggregate into spheroids when grown in nonadherent conditions. In comparison to individual cells, these spheroids more closely represent melanoma tumor architecture. The ability of melanoma cells to expand outward from an initial spheroid into surrounding 3D collagen, mimicking the in vivo collagen-rich dermal layer, is a mark of invasive capacity (27, 28). As differences in gene expression profiles and phenotypic outcomes can arise between cells grown in 2-dimensional versus 3D culture (29, 30), we sought to determine whether high TWIST1 expression is sufficient for invasion in 3D dermal-mimetic conditions. The WM793 cell line is invasive and displays robust spheroid outgrowth in this context (27). Therefore, WM793 spheroids constitutively expressing control or 1 of 3 TWIST1 shRNAs were implanted into 3D collagen and monitored for several days. Strikingly, WM793 cells depleted for TWIST1 displayed significant defects in the ability to begin and sustain spheroid outgrowth compared with control cells (\*, P < 0.01; Fig. 3A). To determine whether TWIST1 expression in a RGP cell line would promote outgrowth, WM35 cells constitutively overexpressing either LacZ (control) or TWIST1 were analyzed. Indeed, significant increases in the spheroid outgrowth of WM35 cells that constitutively overexpress TWIST1 were observed (\*, P < 0.01; Fig. 3B).

ERK1/2–TWIST1–MMP-1 Invasion Axis in Melanoma

Figure 2. TWIST1 is required and sufficient for melanoma cell invasion through Matrigel. WM793 (A) and WM115 (B) cells constitutively expressing control or 1 of 3 independent TWIST1 shRNAs were used. Cells were allowed to invade through Matrigel-coated chambers toward an attractant of full serum media. Counts taken (in triplicate fields of view) from the control shRNA (average set at 100% invasion) were used to calculate percent invasion for all other treatments. Columns, average of 3 independent experiments; bars, SD; scale, 100 μm; \*, P < 0.01. Sbcl2 (C) and WM3211 (D) cells overexpressing either LacZ (control) or TWIST1 were assessed for invasion through Matrigel-coated Boyden chambers as above. Columns, average of 3 independent experiments; bars, SD; scale, 100 μm; \*, P < 0.01.
alteration on cell death and proliferation. At 96 hours post-collagen embedding, spheroids were costained with calcein AM and ethidium bromide to visualize live and dead cells, respectively. No qualitative differences were apparent between any of the spheroids with altered TWIST1 and their control counterparts (Fig. 3C and D, Supplementary Fig. S2). Similarly, at 96 hours, embedded spheroids were subjected to an EdU incorporation assay to evaluate potential differences in S-phase profile. We also analyzed the S-phase profile of adherent cells grown in standard tissue culture dishes. No significant differences were found between control and TWIST1-altered cells in either condition (Fig. 3E and F). From these data, we conclude that the TWIST1-regulated differences in spheroid outgrowth do not result from alterations in cell death or the ability of cells to enter S-phase.

**TWIST1 regulates MMP-1 expression in melanoma cell lines**

In several cancer types, TWIST1 overexpression has been linked to increased tumor invasion and metastasis correlating with features of EMT, a process commonly seen in cell migration during embryonic development (7, 11, 12). Hallmarks of
EMT include loss of cell–cell adhesions and cell polarity through the suppression of epithelial markers (i.e., E-cadherin) and simultaneous acquisition of mesenchymal protein expression (i.e., N-cadherin, β-catenin, Vimentin). Interestingly, the melanoma cell lines used in our studies with altered TWIST1 expression did not display changes in their morphology (data not shown) or EMT protein expression (Supplementary Fig. S3). As a result, an alternative target(s) that is independent of classical EMT must exist in melanoma cells that functions to mediate the effect of TWIST1 expression on altered invasion patterns.

MMPs are key players in the ability of cancer cells to proteolytically degrade the basement membrane and ECM to invade and metastasize. We, therefore, examined whether TWIST1 might alter the expression of MMPs. Quantitative RT-PCR analysis of several MMPs implicated in melanoma progression was done (data not shown). MMP-1 (collagenase-1) was chosen for further analysis as it showed considerable upregulation at the mRNA level after TWIST1 overexpression (\( P < 0.01 \), Fig. 4A and C). MMP-1 is capable of degrading several types of collagen, which is a major component of the dermis in vivo and in the synthetic extracellular matrices used in our in vitro invasion studies in Figs. 2 and 3 (32, 33). In addition, MMP-1 has a well-established role in promoting melanoma cell invasion and metastasis (see Discussion).

In WM793TR cells, TWIST1 shRNA #3, targeting the 3’ untranslated region of TWIST1, elicited a significant decrease in MMP-1 mRNA and secreted protein (\( P < 0.01 \); Fig. 4A). Importantly, reexpression of ectopic (and shRNA-resistant) TWIST1 rescued levels of MMP-1 mRNA and secreted protein in these cells (\( P < 0.01 \); Fig. 4A). Therefore, the effect of TWIST1 on MMP-1 expression is specific and cannot be explained by off-target effects. In addition, overexpression of TWIST1 in the noninvasive cell line Sbccl2 resulted in a more modest but significant increase in MMP-1 mRNA and protein (\( P < 0.05 \); Fig. 4B).

Previous studies found that MMP-1 is regulated downstream of the active RAS-RAF-MEK-ERK1/2 pathway in melanoma (34, 35). We hypothesized that TWIST1 may be a link between activated ERK1/2 and MMP-1 upregulation. Therefore, we assessed the ability for overexpressed TWIST1 to compensate for the loss of active ERK1/2 signaling after treatment with the MEK inhibitor, U0126. Similar to Fig. 4A, TWIST1 overexpression can rescue decreased MMP-1 mRNA and protein expression caused by ERK1/2 pathway inhibition (\( P < 0.05 \), \( P < 0.01 \); Fig. 4C). Taken together, these results show that TWIST1 is a positive regulator of MMP-1 and is a mediator of ERK1/2 signaling to MMP-1 in melanoma.

**TWIST1 alters MMP-1 promoter activity in a dose-dependent manner and directly binds the MMP-1 promoter**

TWIST1 is a transcription factor capable of directly binding E-box consensus sites (5’-CANNTG-3’) to exert transcriptional effects (4, 7). Direct human target genes of TWIST1 remain poorly defined. Their identification is especially intriguing in melanoma given our finding that TWIST1 alteration does not affect EMT genes as it does in other cancer types (7, 11). Several potential binding sites for TWIST1 are present in the MMP-1 proximal promoter (Fig. 5A). We used a 1052bp fragment of the MMP-1 promoter (Fig. 5A) and increasing concentrations of either LacZ or TWIST1 overexpression in dual-luciferase reporter assays in wild-type B-RAF 293FT cells and Sbccl2 cells.
Corresponding to the MMP-1 mRNA/protein expression changes downstream of TWIST1, we found that TWIST1 over-expression increases MMP-1 promoter activity in a dose-dependent manner (*, \( P < 0.05 \); **, \( P < 0.01 \); Fig. 5B and C).

To determine whether TWIST1 was able to bind directly to the MMP-1 promoter, we conducted ChIP of endogenous TWIST1 in parental WM793 cells followed by quantitative PCR of 4 MMP-1 promoter regions (Fig. 5D). Immunoprecipitation was conducted as a negative control for enrichment. Columns, average of 6 independent experiments; bars, SD; *, \( P < 0.05 \); **, \( P < 0.01 \). D, in parental WM793 cells, ChIP followed by quantitative PCR was conducted to determine if TWIST1 binds directly to the MMP-1 promoter. V5 epitope immunoprecipitation was conducted as a negative control for enrichment. Columns, average of 6 independent experiments; bars, SD; *, \( P < 0.05 \); **, \( P < 0.01 \). E, a biotinylated oligonucleotide pulldown assay was conducted with whole cell protein lysates from parental WM793 cells. A pulldown without oligonucleotide was conducted as a negative control. Following the pulldown, Western blot analyses were conducted to determine TWIST1 enrichment relative to 10% input. F, oligonucleotide pulldowns were repeated for MMP-1 E-box #5 without nonlabeled competitor or in the presence of 5-, 10-, and 20-fold excess of nonlabeled E-box #5 competitor. Also, a biotinylated mutant E-box #5 oligonucleotide (4 mutant nucleotides) was used to determine the specificity of the interaction.

MMP-1 is able to rescue defects in spheroid outgrowth resulting from TWIST1 ablation

Previous studies have established that changes in MMP-1 expression in melanoma cells lead to alterations in collagen production. We found that over-expression of MMP-1 in melanoma cells results in increased collagen degradation and enhanced spheroid outgrowth. These findings suggest that MMP-1 plays a critical role in regulating the extracellular matrix and cell motility in melanoma cells.
degradation abilities, invasion in vitro, and metastasis in vivo (33, 36–39). We sought to determine whether forced expression of MMP-1 could rescue defects in melanoma spheroid outgrowth resulting from TWIST1 ablation.

For these studies, we used WM793TR cells that inducibly express either control shRNA or TWIST1 shRNA #3. We then engineered each of these cell lines to overexpress either a LacZ control or MMP-1 protein upon doxycycline addition (Fig. 6A). WM793TR spheroids, generated in the presence or absence of doxycycline, were implanted into 3D collagen and doxycycline treatments maintained. WM793TR spheroids that were grown in the absence of doxycycline displayed spheroid growth typical of a VGP cell line with no significant differences apparent between the variants (Fig. 6B and C, left; Supplementary Fig. S4). The induction of TWIST1 shRNA #3 + LacZ in WM793TR cells caused significant defects in spheroid outgrowth at all time points examined, as was previously established in Fig. 3 (*, P < 0.05, **, P < 0.01; Fig. 6B and C, right; Supplementary Fig. S4). Importantly, WM793TR-TWIST1 shRNA #3 cells engineered with a rescue of MMP-1 expression showed a significant increase in spheroid outgrowth area as compared with those cells with TWIST1 depletion alone (**, P < 0.001). From these studies, we can gather that MMP-1 is a major effector used by TWIST1 to alter invasion in melanoma.

Discussion

Our results provide evidence that active ERK1/2 signaling promotes upregulation of TWIST1, which in turn positively regulates the known invasion protein, MMP-1 (Fig. 7). Mutations that lead to hyperactivation of the RAS-RAF-MEK-ERK1/2 pathway occur in 30% of all malignancies and are considered in many contexts to be early initiators of neoplasia (40). Melanoma is an optimal model system to study the downstream effects of hyperactive ERK1/2 signaling, as it occurs in approximately 90% of these patients and leads to evasion of apoptosis and increased tumor growth and invasion (19). The mechanisms stemming from constitutively active ERK1/2 signaling leading to increased tumor invasion have yet to be fully established. In this study, we present clear evidence that RAS-RAF-MEK-ERK1/2 signaling positively regulates TWIST1. Through pharmacologic inhibition, siRNA/shRNA ablation, and protein rescue experiments, we show that active ERK1/2 signaling tightly controls TWIST1 protein expression through alterations in transcript levels in mutant B-RAF cells. These data are consistent with microarray data previously published by Shields and colleagues (41). We have also found changes in TWIST1 in 4 mutant N-RAS melanoma cell lines (Supplementary Fig. S5); however, because of variability in the timing of downregulation and effects on transcript levels (data not shown), we have not further examined the mechanism in this subgroup. Thus, we cannot rule out ERK1/2 dependent, posttranscriptional regulation of TWIST1 in these cells.

Melanoma is also a model for the invasive process, as the highly metastatic nature of this tumor type leads to chemotherapy resistance and its classification as the deadliest form of skin cancer. Aberrant invasion is one of the main
activate protease activated receptor 1. Invasion associated with its ability to degrade collagen in the ECM and MMP-1 and its cleavage into an active form, MMP-1 promotes cancer cell
downstream of ERK1/2 require further investigation. Through our studies,
processes by which cancer cells evade tissue boundaries to achieve colonization at metastatic sites. To that end, many
cancer cells will exploit developmental mechanisms of motility and migration. The TWIST1 protein has been well
studied in development as a main molecule responsible for cell migration from the neural crest during embryogenesis through EMT mechanisms. Before this study, TWIST1 was known to be upregulated in melanoma and associated with poor prognosis (8, 14); however, the mechanism and role of elevated TWIST1 expression in melanoma has remained unexplored. Here, we report that TWIST1 positively regulates melanoma cell invasion. The promotion of invasion by TWIST1 was observed in 2 3D invasion assays, 1 representing invasion through a basement membrane and the other mimicking the dermal microenvironment following invasion through the basement membrane. Despite previous reports of TWIST1 interaction with the p53 pathway (8, 31), cell-cycle or cell death changes were not involved in the invasion phenotypes that we observed.

Interestingly, TWIST1 expression seems to be the highest in VGP melanoma cell lines, which are derived from tumors that have invaded vertically into the dermis and can eventually lead to metastasis. Within our mutant B-RAF cell line panel, all metastatic melanoma cells overexpress TWIST1 compared with melanocytes, but have reduced expression compared with mutant B-RAF VGP cell lines. This finding seems to contrast with previous studies where TWIST1 immunohistochemical staining was more prominent in metastatic melanoma tissue cores compared with primary melanomas (14). One potential explanation for this disparity is that the tissues in the microarray used in this study were not separated by genotype. In our cell line panel, TWIST1 was more highly expressed in mutant B-RAF cell lines in comparison to similar stage wild-type B-RAF cell lines. In the absence of organization by genotype, the prominence of TWIST1 expression in mutant B-RAF VGP tumors might have been obscured. In support of our data, previous work by Weinberg and colleagues describes the requirement for TWIST1 in early metastatic steps of invasion and intravasation and not in later steps (7). For these studies, they used 4 cell lines derived from the same tumor with differing metastatic potential and found that TWIST1 RNA/protein expression is higher in the cell lines that can only invade and intravasate, which is in agreement with our data. We hypothesize that once melanoma invasion and intravasation is accomplished, the selective pressure for TWIST1 expression is removed and therefore maintenance of high TWIST1 expression is no longer necessary.

We further provide a mechanistic basis for the effects of TWIST1 on melanoma invasion. Surprisingly, TWIST1 did not alter EMT-associated proteins as has been well documented in other cancer types (7, 11, 12). Instead, we provide considerable evidence that TWIST1 positively regulates MMP-1 mRNA leading to substantial changes in secreted protein levels. Through luciferase reporter assays, ChIP, and in vitro oligonucleotide pulldown assays, TWIST1 regulation of MMP-1 was found to be dose dependent and likely occurs through direct contact at multiple E-box sites within the MMP-1 proximal promoter. The expression of various MMPs has been linked to tumor invasion in a number of systems through matrix-degrading activities (33, 38). Specifically, MMP-1 (collagenase-1) plays a vital and substantial role in melanoma cell invasion most likely as a result of the collagen-rich dermal environment surrounding these cells (33). Melanoma cells that are invasive express much higher levels of MMP-1 in comparison to noninvasive melanomas (36). Furthermore, depletion of MMP-1 expression in invasive melanoma cells results in decreased invasion through chambers coated with type I collagen, type IV collagen, or Matrigel (33, 39). Also, forced expression of MMP-1 in RGP melanoma cells increased collagen degradation in vitro, and tumor growth and metastasis in vivo (39). We show that cells depleted of TWIST1, but with forced MMP-1 expression, display a restored ability to invade into 3D collagen. These
effects are likely ECM protein specific, as changes in TWIST1 expression significantly alters the ability of melanoma cells to invade through and migrate toward collagen type 1 in Boyden chamber assays, but does not significantly affect migration toward other ECM components such as fibronectin and gelatin (Supplementary Fig. S6). Therefore, MMP-1 is likely to be a major mechanism that TWIST1 uses to increase invasion of melanoma cells through the collagen-rich dermis. Of note, TWIST1 was recently found to be critical in the formation of invadopodia, which degrade the ECM through protease action, in mammary tumor models (42). In light of our studies, it is intriguing to postulate that TWIST1 might increase both the formation and activity of invadopodia through upregulation of MMP-1.

MMP-1 was recently highlighted as target of RAS-RAF-MEK-ERK1/2 signaling in melanoma (34, 35). Here, we uncover a novel mediator of this signaling with evidence of an ERK1/2–TWIST1–MMP-1 signaling axis. B-RAF mutations are common in preneoplastic nevi and, therefore, are considered by many to represent an initiation step in melanoma progression (43). While the majority of common melanocytic nevus harbor mutant B-RAF, only 23% of those display phosphorylated ERK1/2. However, 54% of B-RAF–mutant atypical nevi, which are those that represent an increased risk of developing into melanoma, have active ERK1/2 (44). One mechanism atypical nevi may use to progress into melanoma might be through removal of negative checks in the ERK1/2 pathway leading to upregulation of TWIST1 and subsequent increases in MMP-1. Indeed in the clinic, melanoma patients with higher MMP-1 serum levels had significantly shorter time to progression as compared with those patients with lower MMP-1 serum levels (45). MMP inhibitors failed in clinical trials for melanoma and other cancers largely because of the enrollment of patients with extremely advanced disease (46). Because of the limited availability of treatment options for metastatic melanoma patients, it might be of value to explore the use of MMP-1 inhibitors as prophylactics in the premetastatic stages of melanoma disease.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: M.B. Weiss, A.E. Aplin
Development of methodology: M.B. Weiss, E.V. Abel, M.M. Mayberry, K.J. Basile
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.B. Weiss, M.M. Mayberry, K.J. Basile, A.C. Berger
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.B. Weiss, M.M. Mayberry, A.E. Aplin
Writing, review, and/or revision of the manuscript: M.B. Weiss, E.V. Abel, A.C. Berger, A.E. Aplin
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.B. Weiss, A.C. Berger
Study supervision: M.B. Weiss

Acknowledgments
The authors thank Drs. Meenhard Herlyn for WM melanoma cell lines and Dave Solt for SK-MEL-32 and SK-MEL-207. The authors thank Gideon Bollag (Flexizyme Inc., Berkeley, CA) for PLX4720/PLX4032. The authors thank Dr. Yongping Shao, Curtis Kugel, and Kaithlyn Le for reagents, and Drs. Ethan Abel and Yongping Shao for manuscript review.

Grant Support
This work is funded by the American Cancer Society John W. Thatcher, Jr. Postdoctoral Fellowship in Melanoma Research (PF-11-240-01-DDC), and NIH RO1s CA125103 and GM67893. Dr. Ethan Abel and Kevin Basile were supported, in part, by fellowships from the Joanna M. Nicolay Melanoma Research Foundation. The TJU KCC core facilities are supported by the NCI Support Grant IP30CA66036.

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 March 20, 2012; revised September 21, 2012; accepted October 8, 2012; published OnlineFirst December 7, 2012.
Weiss et al.


27. Smalley KS, Haass NK, Bradford PA, Lioni M, Flaherty KT, Herlyn M. Multiple signaling pathways must be targeted to overcome matrix resistance in cell lines derived from melanoma metastases. Mol Cancer Ther 2006;5:1136–44.


TWIST1 Is an ERK1/2 Effector That Promotes Invasion and Regulates MMP-1 Expression in Human Melanoma Cells

Michele B. Weiss, Ethan V. Abel, Melanie M. Mayberry, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-12-1033

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2012/10/22/0008-5472.CAN-12-1033.DC1

Cited articles
This article cites 46 articles, 16 of which you can access for free at:
http://cancerres.aacrjournals.org/content/72/24/6382.full#ref-list-1

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/72/24/6382.full#related-urls

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

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

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/72/24/6382.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.