Introduction
Melanoma is one of the most virulent human cancers due to its tendency to metastasize and resistance to conventional anticancer therapies. One key factor responsible for treatment failure relates to tumor heterogeneity, particularly the cell populations possessing stem cell-like properties (1). Several putative stem cell markers have been reported in melanoma to date (2–9). Among these, CD133 (human prominin 1), a transmembrane pentaspan glycoprotein found in stem and early progenitor cells of varying histogenesis, and ABCB5, an ATP-binding cassette (ABC) efflux transporter responsible for melanoma chemoresistance, are the best characterized. Monzani and colleagues (5) showed that human melanoma samples contain CD133+ fractions, which confer multipotent differentiation in vitro, as well as tumorigenic growth in vivo. CD133 downregulation in melanoma cells led to reduced motility and metastasis (10). Frank and colleagues (2) reported that downregulation in melanoma cells led to reduced motility and in vitro tumorigenesis.

Abstract
Tumor cell subpopulations that express cancer stem cell markers such as CD133 (prominin1) or ABCB5 are thought to be crucial for tumor initiation and heterogeneity, but their biological significance in melanoma has been controversial. Here, we report that CD133+ and ABCB5+ subpopulations are colocalized in melanomas in perivascular niches that contain CD144 (VE-cadherin)+ melanoma cells forming vessel-like channels, a phenomenon termed vasculogenic mimicry (VM). RNAi-mediated attenuation of CD133 established its critical function in morphogenesis of these perivascular niches as well as in melanoma tumorigenicity. Niche-associated genes CD144 and ABCB5 were downregulated in tumors derived from CD133 knockdown (KD) melanoma cells compared with controls. CD133KD cells also lacked the ability to form CD144+ VM-like channels in a manner that was associated with a depletion of the ABCB5+ cell subpopulation. Finally, CD133KD cells exhibited poorer tumor growth in vivo. Taken together, our findings corroborate models in which CD133+/ABCB5+ melanoma cells reside in a complex anastomosing microvascular niche that encompasses CD144+ VM channels as well as authentic endothelial cell-lined blood vessels. Further, they indicate that CD133+ cells act as stem-like cells, which drive tumor growth by promoting VM and the morphogenesis of a specialized perivascular niche in melanoma.

CD133+ Melanoma Subpopulations Contribute to Perivascular Niche Morphogenesis and Tumorigenicity through Vasculogenic Mimicry
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Tumor and Stem Cell Biology

CD133+ melanoma cells co-express CD133 in vitro and exhibit self-renewal, differentiation and tumor-initiating capacities in vivo. Furthermore, direct targeting of the ABCB5+ melanoma subpopulation using a monoclonal antibody (mAb) resulted in Ah-dependent cell-mediated cytotoxicity exerting tumor-inhibiting effects (3). Findings from these earlier studies are in keeping with the traditional "hierarchical model" of cancer stem cell (CSC) hypothesis, where the marker-expressing fractions are endowed with self-renewal capacity and sufficient to reconstitute/repopulate the tumor by generating both progenitor marker-positive daughter CSC and marker-negative differentiated progenies through asymmetrical division. However, the recent identification of tumor-initiating potential within CD133− subsets in brain, colon, and lung cancers as well as melanoma raises questions on the biological relevance of these putative marker-expressing subsets (11–14).

Although traditionally CSC are defined mainly by their ability to self-renew and differentiate, mounting evidence now suggests that like normal stem cells, CSC reside in specialized compartments, known as the "niche," which provide the essential cues for cell fate determination (15–17). For example, a recent report by Calabrese and colleagues (18) localized brain tumor initiating cells (BTIC) to the perivascular microenvironment, the so-called "perivascular niches." Disruption of such vascular niches ablates the brain tumor initiating population, resulting in growth arrest (18). Rather than passively, functionally depend on the supporting blood vessels within the niche, it is now clear that the BTIC actively and directly participate in tumor vascularization through transendothelial

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differentiation (19, 20). Despite advances in identifying markers for melanoma-initiating cells (MIC) and subsequent characterization of different MIC subsets, their microenvironmental niches have not been defined.

To test the biological significance of CD133+ melanoma subsets, we: (1) examined the expression of CD133+ subsets in vitro and in vivo, (2) characterized their microenvironmental niches in the context of tumor microcirculation, comprised of not only authentic blood vessels but also vasculogenic mimicry (VM) channels formed by transdifferentiated melanoma cells (21–23), and (3) explored their functional role with regard to tumor growth and niche maintenance through loss-of-function analysis using lentiviral shRNA. Defining and characterizing the MIC niche and molecular mechanisms through which different resident cell types communicate offers a novel opportunity to therapeutically eliminate MIC directly or indirectly by targeting the stromal codependence.

Materials and Methods

Cell culture

Isogenic melanoma cell lines derived from different disease stages of tumor progression (obtained from Dr. M. Herlyn at the Wistar Institute, Philadelphia, PA) were cultured as previously described (24). These were composed of primary vertical growth phase (VGP) melanoma cell lines WM115 and WM983A, and their metastatic counterparts WM239A and WM983C, respectively, and metastatic melanoma variants, WM164, WM1617, 1205Lu, and SK-MEL-5. Metastatic melanoma cell lines A375 and its highly invasive variant, A375 P-5 (obtained from Dr. M.J.C. Hendrix at the Northwestern University, Chicago, IL; ref. 25) were maintained as previously described (25). All cell lines were tested using the PowerPlex 18D system (Promega BioSciences).

Flow cytometry

Surface CD133 expression on melanoma cells was analyzed by flow cytometry using phycoerythrin (PE)-conjugated CD133/1 clone AC133 antibody (Miltenyi Biotec), PE-conjugated isotype control mAb (Abcam) served as a control. 7AAD (MP Biomedicals) was added to samples prior to flow analysis to facilitate discrimination against dead cells. The expression profile was analyzed with acquisition of fluorescence emission at the FL2 (PE) spectrum on LSR II (BD Biosciences) using FACS Diva software (BD Biosciences).

Immunohistochemistry

Dewaxed formalin-fixed and paraffin-embedded (FFPE) melanoma tissue sections were subjected to antigen-retrieval using a Pascal pressure chamber (Dako) followed by immunohistochemical staining using rabbit anti-CD133 (Abcam), goat anti-ABC5b (Abcam), or isotype-matched negative control primary antibodies (Abcam), and peroxidase-conjugated goat anti-rabbit or horse anti-goat secondary antibodies (Vector Laboratories). Immunoreactivity was visualized by NovaRed peroxidase substrate (Vector Laboratories).

Patient tissue samples

Decoded patient samples, including 7 benign nevi, 7 primary cutaneous melanomas (ranging from Clark level III-IV), and 7 metastatic melanomas (from various tissue sites, such as lung, lymph node, and cutaneous metastases), were obtained from the archive at the Department of Pathology, Brigham and Women's Hospital according to Institutional Review Board-approved protocol #2009P001579). No prior treatment history of these individuals was available.

Immunofluorescence

Frozen melanoma xenograft sections or dewaxed FFPE patient tissue sections following antigen retrieval were subjected to double indirect immunofluorescence (IF) using standard procedures. The primary antibodies used in this study are rabbit anti-hCD144 (human-specific: Cell Signaling Technology), rabbit anti-CD144 (reactive to human and mouse, Abcam), goat anti-GFP (Novus Biologicals), rabbit anti-CD133 (Abcam), rat anti-mouse CD31 (BD Biosciences), mouse anti-β3 integrin (SAP, gift from Dr. M. Herlyn), and mouse antihigh molecular weight proteoglycans (ME31.3; gift from Dr. M. Herlyn). The secondary antibodies used are FITC-conjugated donkey anti-rabbit IgG (Accurate Chemical & Scientific Corporation), FITC-conjugated donkey anti-goat (Jackson Immunoresearch), AlexaFluor 594-conjugated donkey anti-rabbit, AlexaFluor 594-conjugated donkey anti-rat IgG, and AlexaFluor 488-conjugated goat anti-mouse IgG1 (Invitrogen) antibodies. Isotype-matched rabbit, goat, or rat immunoglobulin was used in place of the primary antibody for control. Sections were mounted with Vectashield containing DAPI (Vector Laboratories) and inspected under a BX51/BX52 fluorescence microscope (Olympus) or DSU confocal microscope (Olympus).

In situ hybridization

In situ hybridization was carried out as previously described (26). The sequences used for human CD133 probes are listed as follows: CD133 sense strand 5'-GACCCGAAGCTCCCATATAAGC-3'; CD133 antisense strand 5'-GACCCCAAGGTGAGCAT-3'. The ABC5B probes were previously described (27). The probes were labeled with digoxigenin using DIG RNA labeling kit (Roche).

CD133 knockdown in melanoma cells by lentiviral shRNA

Lentiviral vectors were generated by cotransfecting pLKO.1-CD133 (Sigma), containing shRNA against human CD133, or nontarget control shRNA (Sigma) with packing plasmids VSVG and pCMV-AR8.2 (Sigma) into 293T packing cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Lentiviral supernatants were used to infect WM1617 melanoma cells. Stable transfectants were selected with 1 μg/ml puromycin for a period of 7 days.

Western blotting

Cell lysates and xenograft tissue homogenates were extracted in lysis buffer and quantified by a BCA protein assay kit (Pierce) as previously described (28). Bovine aortic endothelial cell (BAEC, Cell Signaling Technology) and A375 lysates
were included as a positive control for CD144 and ABCB5, respectively. Equal amounts (10 to 50 μg) of protein were subjected to electrophoresis, followed by probing with rabbit anti-CD133 (Abcam; Western blot analysis for cell lysates) at 1 μg/mL or mouse anti-CD133 (Miltenyi Biotec, clone W6B3C1; Western blot analysis for xenograft homogenates) at 1:200; rabbit anti-CD144 (Cell Signaling Technology) at 1:1,000; mouse anti-β actin (Abcam) at 1:5,000. Immunoreactive bands were visualized by SuperSignal West Pico Chemiluminescent Substrate (Pierce). Densitometry measurements were done using Image J software (National Institutes of Health), where β-actin served as a loading control.

**Melanoma xenografts**

For characterization of the perivascular niche, 3 × 10^6 A375, A375GFP (A375 stably expressing GFP mediated by lentiviral gene transfer), SK-MEL-5, and WM1617 melanoma cells were injected subcutaneously in the dorsal skin of each SCID mouse (CB17; Taconic Laboratory). Melanoma xenografts were harvested when tumors reach 1 cm³ and subjected to histopathologic analysis.

**In vivo tumorigenicity**

For the effect of CD133 silencing on tumorigenicity, 2 × 10^5 WM1617 melanoma cells infected by control and CD133 shRNA lentiviral constructs were injected subcutaneously in the dorsal skin of each SCID mouse (CB17; Taconic Laboratory; 5 mice per condition). In another separate experiment, 3 × 10^6 control and CD133 KD WM1617 melanoma cells were injected per mouse (7 mice per condition) to ensure the generation of sizable CD133 KD xenografts for various analyses, including immunohistochemistry, IF, and real-time quantitative RT-PCR as described above. Tumor volume was monitored and determined as the volume of ellipsoid: 4/3 π (width/2 × length/2 × height/2). Statistical analyses were carried out using ANOVA following log transformation.

**Real-time quantitative RT-PCR**

RNA from frozen tumors was extracted and reverse transcribed into cDNA using μMACS One-step cDNA Kit (Miltenyi Biotec) according to the manufacturer's instructions. Real-time quantitative PCR (q-PCR) was subsequently carried out on a 7300 Real-Time PCR System (Applied Biosystems) using human-specific primers, CD133 (Hs00195682_m1), ABCB5 (Hs00698751_m1), or CD144 (Hs00174344_m1). All samples were run in triplicate. The actin housekeeping gene was used for normalization and data analyzed using 2^−ΔΔCt method (29).

**Results**

**CD133 expression in melanoma correlates with tumor progression in vivo**

Using flow cytometry, we identified CD133^+ fractions in 7 melanoma cell lines, including WM164, WM1617, A375P-5, and

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**Figure 1.** Expression of CD133 subsets in melanoma. A, in vitro expression of CD133 in cultured melanoma cell lines was detected by PE-conjugated anti-CD133 (Miltenyi Biotec) using flow cytometry. □, VGP melanoma cell lines; ■, metastatic melanoma (Met). B, CD133 expression in patient samples was examined by IF. Seven samples of each stage of melanoma progression were examined by IF. Seven samples of each stage of melanoma progression were examined and the percentage of positive cells was recorded by examining 10 randomly chosen ×400 fields. C, double IF using antibodies directed to melanoma-associated antigen β3 integrin and high molecular weight proteoglycans (green, left panels) verified that the CD133-expressing cells (red, middle panels) in patient samples were indeed melanoma cells. Magnification, ×400; scale bar, 25 μm.
2 isogenic cell pairs: WM115 (VGP)/WM239A (Met) and WM983A (VGP)/WM983C (Met), out of a total of 9 (7/9) in vitro. The CD133\(^+\) subsets in the positive cell lines range from 0.2% to 76.3% (Fig. 1A). Two metastatic melanoma cell lines 1205Lu and A375 exhibited no detectable CD133. CD133 expression in patient samples was also examined by IF. Seven lesions each of different stages of melanoma progression, including ordinary nevi, primary melanomas, and metastatic melanomas, were analyzed. We found that the average percentage of the CD133\(^+\) fraction rising step-wise from 0% in nevi to 24% in metastatic lesions, whereas the percentage of samples containing the CD133\(^+\) fraction increasing from 0 of 7 in nevi to 7 of 7 in metastatic lesions, indicating that the CD133\(^+\) subset is more prevalent as the disease progresses (Fig. 1B). To validate that the CD133\(^+\) cells identified in the patient samples were indeed melanoma cells, double IF was used using antibodies directed to melanoma-associated antigens (MAA), such as \(\beta3\) integrin subunit or high molecular weight proteoglycans (Fig. 1C). Taken together, CD133 expression correlates with tumor progression in vivo, though no obvious correlation with tumor progression was observed in vitro because CD133 expression was detected in both primary melanoma cell lines (2/2: e.g., WM115 and WM983A) but not in all metastatic melanoma cell lines screened (5/7).

CD133\(^+\)/ABCB5\(^+\) subsets coincide with CD144\(^+\) areas of VM: the perivascular niche

To explore the tissue distribution of CD133\(^+\) subsets in melanoma, we conducted immunohistochemistry (IHC) and in situ hybridization (ISH) in melanoma xenografts derived from WM1617 (not shown), A375 (Fig. 2), and SK-MEL-5 melanoma cells (not shown). We found that CD133\(^+\) fractions coincide with ABCB5\(^+\) subsets, and are arranged in a branching network pattern identical to the PAS\(^+\) VM channels (Fig. 2A). To further characterize VM in vivo, we analyzed the tumor vasculatures in relationship to the CD133\(^+\) subsets in GFP-labeled A375 melanoma xenografts by multilabel IF. Using CD144 as a marker for VM, we identified circulatory networks composed of CD144\(^+\)/GFP\(^+\) melanoma cell-lined VM channels, CD144\(^+\)/GFP\(^+\)/mCD31\(^-\) EC-lined mouse blood vessels (BV, red arrowhead), and mosaic vessels (MV) partially lined by both CD144\(^+\)/GFP\(^+\) VM-engaging melanoma cells (orange arrow) and CD144\(^+\)/GFP\(^+\)/mCD31\(^-\) ECs (Fig. 2A). CD133\(^+\)/ABCB5\(^+\) subsets coincide with CD144\(^+\) areas of VM, indicating the perivascular niche in consecutive sections of WM1617 xenograft. Each asterisk indicates the same corresponding blood vessel in both panels. Scale bar, 50 \(\mu\)m.
vessels (BV), and mosaic vessels (MV) partially lined by both CD144+/GFP+ channel-forming melanoma cells and CD144+/GFP+ mouse ECs (Fig. 2B). Interestingly, CD144+ VM-engaging melanoma cells (Fig 2C, right panel) are confined in areas in close proximity to CD31+ mouse BV (Fig. 2C, left panel) in consecutive tissue sections. Furthermore, IF localizes CD133+ subsets (Fig. 2D, left panel) to CD144+ VM fractions (Fig. 2D, right panel) intimately associated with CD31+ mouse BV in a "perivascular pattern." Collectively, these data suggest that CD133+/ABCB5+ subsets reside in a complex anastomosing microvascular niche encompassing CD144+ melanoma cell-lined VM channels, mosaic vessels partially lined by CD144+ VM-engaging melanoma cells and endothelial cells, and authentic blood vessels.

**CD133 silencing in melanoma by lentiviral shRNA**

To elucidate the functional role for CD133, we generated stable CD133 KD in WM1617 melanoma cells using lentiviral shRNA. Western blotting revealed over 90% knockdown efficiency at the protein level achieved by CD133 shRNA both in vitro and in vivo (xenografts) compared with the control harboring nontarget shRNA (Fig. 3A). IHC (Fig. 3B) further verified downregulation of CD133 in vivo, as only the nontarget control but not CD133 KD xenografts retained the perivascular niche VM-like pattern of CD133 expression.

**CD133 KD downregulates CD144 and ABCB5, attenuates VM-like melanoma channel formation, and retards tumor growth**

When we analyzed CD133 KD xenografts by real-time qRT-PCR using human-specific primers, expression profiling showed concurrent downregulation of CD144 and ABCB5 in CD133 KD xenografts compared with their nontarget control counterparts (Fig. 4A). IF staining revealed that CD133 KD xenografts exhibited attenuated CD144+ VM-like melanoma channel formation, whereas the control xenografts retained CD144+ VM channels in close association with CD31+ authentic blood vessels (Fig. 4B).

IHC analysis (Fig. 4C) also confirmed the depletion of vascular niche-associated ABCB5+ subsets in CD133 KD xenografts. Attempts to further validate CD144 downregulation in CD133 KD xenografts using Western blotting (BAEC lysate included as a positive control) were unsuccessful due to sensitivity issues given the minute fraction of the CD144+ subset (Fig. 4D, top panel; note that CD144 signals were undetectable both in the control and KD xenografts despite maximal loading and prolonged overnight exposure). Western blotting (Fig. 4D, middle panel; using A375 cell lysate as a positive control); however, confirmed downregulation of ABCB5 at the protein level in CD133 KD xenografts. Furthermore, in 2 separate tumorigenicity assays, CD133 KD significantly (p < 0.05) retarded tumor growth compared with the nontarget control in vivo (Fig. 4E), whereas in conventional monolayer growth as well as soft agar assays, both CD133 KD and control cells exhibited comparable growth rates and clonogenicity in vitro (Supplemental Figure 1).

**Discussion**

The recognition of cancers as heterogeneous at both the genomic and cellular levels has prompted intense investigation of cancer cell subpopulations. Of particular interest are the subpopulations that exhibit stem cell-like properties, as these may be recalcitrant to traditional cancer therapies, and therefore, contribute to tumor recurrence and metastasis. Although potential cancer cell populations expressing stem cell-like biomarkers in melanoma are studied extensively (2–8, 30), the microenvironment that supports their functional integrity has received scant attention. Our data reveal that CD133+/ABCB5+ subpopulations coincide with fractions of CD144+ melanoma cells forming VM-like channels, where high-density EC-lined blood vessels also reside (Fig. 2). Parallel to our findings, Frank and colleagues (27) recently reported that the ABCB5+ melanoma fractions preferentially express VM markers, CD144 and Tie-1. Furthermore, increasing evidence now supports the notion that stem cell marker-expressing tumor

![Figure 3. CD133 silencing in melanoma using lentiviral shRNA. A, cell lysates and xenograft homogenates prepared from WM1617 melanoma cells stably transfected with lentiviral shRNA against human CD133 and nontarget control (Cnt.) were subjected to Western blotting. β-actin served as internal loading control. Greater than 90% CD133 KD efficiency was achieved in vitro and in vivo compared with the nontarget control as determined by densitometry. WT, parental WM1617 nontransfectant. B, immunohistochemistry confirmed CD133 silencing in vivo in WM1617 melanoma xenografts. Note that CD133 KD xenografts are devoid of CD133 expression, whereas those of nontarget control retain constitutive CD133 expression in the perivascular niche. b.v., blood vessel. Magnification, ×100 (inserts, ×400); scale bar, 50 μm.](cancerres.aacrjournals.org)
In this regard, BTIC-derived CD31 through transdifferentiating towards endothelial cell lineage. That BTIC are also able to contribute directly to vascular niche cells. In addition, several laboratories have recently showed of vascular niches through paracrine stimulation of endothelial (SDF-1; ref. 36) have been shown to contribute to the formation VEGF (19, 20, 35) and CXCL12 or stromal derived factor-1 (15, 33, 34). For example, BTIC-associated factors, such as communicance exist between the BTIC and the perivascular niche now indicates that complex reciprocal and coordinated com-

**Figure 4.** Consequences of CD133 KD in WM1617 melanoma. A, expression profiling by qRT-PCR revealed concomitant CD144 and ABCB5 downregulation in CD133 KD xenografts. □ nontarget control; ■ CD133 KD. B, IF analysis showed attenuated CD144 VM-like channel formation in perivascular niche in CD133 KD xenografts. Magnification, ×200; scale bar, 50 μm. C, immunohistochemistry showed depletion of perivascular ABCB5 subsets in CD133 KD xenografts. Magnification, ×200; scale bar, 50 μm. D, Western blotting for CD144 and ABCB5 expression in WM1617 xenografts. Unfortunately, despite maximal loading (40 μg protein per lane) and prolonged exposure (overnight), the expression of CD144 was under the detection limit both in the nontarget control and in the CD133 KD xenografts (BAEC lysate was included as a positive control). On the other hand, ABCB5 downregulation in CD133 KD xenografts was confirmed by Western blotting (A375 cell lysate served as a positive control). E, tumorigenicity assays revealed that CD133 KD in WM1617 melanoma cells resulted in significant growth inhibition in vivo (upper panel, n = 5; 2 × 10⁶ cells/mouse; lower panel, n = 7; 3 × 10⁶ cells/mouse). *P < 0.05.

subpopulations are closely associated with VM. For example, stem cell marker CD271 is preferentially expressed by the VM-forming uveal melanoma cells (31), and the CD133⁻ subset of triple-negative breast cancer correlates with VM-forming capability (32).

The close spatial association between CD133⁻/ABCB5⁺ subsets and the blood vessels is reminiscent of the perivascular niche concept of BTIC, where the tumor vasculature serves as a privileged environment that provides the cues to preserve the functional integrity of BTIC. However, the flow of signals within the niche is not unidirectional. Compelling evidence now indicates that complex reciprocal and coordinated communications exist between the BTIC and the perivascular niche (15, 33, 34). For example, BTIC-associated factors, such as VEGF (19, 20, 35) and CXCL12 or stromal derived factor-1 (SDF-1; ref. 36) have been shown to contribute to the formation of vascular niches through paracrine stimulation of endothelial cells. In addition, several laboratories have recently showed that BTIC are also able to contribute directly to vascular niche through transdifferentiating towards endothelial cell lineage. In this regard, BTIC-derived CD31 ECs were found to incorporate into the tumor vasculature as shown by fluorescent lineage tracer and identification of cancer cell-specific molecular signatures (20, 35, 37). Parallel to this, we observe that the melanoma vascular niche encompasses a complex vascular network consisting not only of authentic EC-lined blood vessels, but also of VM channels formed by CD144⁺ transdifferentiated melanoma cells and mosaic vessels (Fig. 2B). Interestingly, unlike BTIC-derived ECs, the CD144⁺ VM-engaging melanoma cells do not express the conventional EC marker, CD31. Nevertheless, the plasticity of tumor cells to differentiate toward endothelial lineage is strikingly similar (21, 22).

Although the role of CD133 as a CSC marker is supported by multipotency in vitro and tumorigenicity in vivo in various human cancers, tumor-initiating potentials within CD133⁻ fractions have been reported in brain, colon, and lung cancers (11–14) as well as in melanoma (38). In this study, we found that A375 melanoma cells, despite harboring a comparable number of CD133⁻ cells in xenografts (Fig. 2), are negative for CD133 in vitro (Fig. 1A). Similar induction of CD133⁻ subsets from CD133⁻ cells in vivo have been observed by others (9, 13), suggesting that tumor "stemness" is a dynamic process and progenitor marker-negative cells may evolve into CSCs through proper environment cues or accumulating genetic alterations, the so-called stochastic model of CSC hypothesis.
(39, 40). The plasticity of stemness in cancer cells is further supported by recent reports where CSC-like phenotype can be induced in non-CSC in response to ectopic expression or upregulation of stem cell-associated transcription factors (41–42). Because of the dynamic nature of the tumor stemness, single marker-based targeting may have limited benefit and the success of CSC-targeted therapy may rely on niche-dependent strategies (15, 33, 43). Adding to the complexity is the notion of a modified “hierarchical” model of CSCs, which postulates that several different types of CSCs may exist in a given tumor, yet a subset of these are better suited to survive and proliferate in a given microenvironment. This scenario provides an explanation for the lack of complete growth inhibition in CD133 KD cells in vivo (Fig. 4E), and again potentially be a disincentive for developing therapeutics targeting a specific class of cell surface markers (44, 45).

Although the precise cellular function of CD133 is largely unknown, downregulation of CD133 in melanoma xenografts resulted in growth retardation (Fig. 4E), attenuated CD144+ VM channel formation and depleted ABCB5+ subsets within perivascular niche (Fig. 4A to D), and reduced cell motility and metastasis (5), supporting its role in tumor growth and maintenance. Thus, our data suggest that CD133+ subsets may contribute to perivascular niche morphogenesis and tumorigenesis through coordinating CD144+ VM formation. Interestingly, CD133 KD does not appear to render growth disadvantage either in conventional monolayer culture or in soft agar in vitro (Supplemental Figure 1), further highlighting the biological importance of the niche microenvironment in the phenotypic/functional rendering of MIC subpopulations.

The underlying mechanisms through which the CD133+ subsets contribute to VM in melanoma are unclear; however, several molecular pathways that regulate VM have recently been identified (46). For example, VEGF-A signaling upregulates VM-associated genes, such as CD144 and EphA2, in stem cell marker-expressing tumor populations (27, 47) and VEGFR-1 knockdown inhibits VM-forming ability of ABCB5+ melanoma cells (27). Expression of pigment epithelium-derived factor (PEDF) inversely correlates with VM, and PEDF downregulation in nonaggressive melanoma cells induces the VM phenotype (48). Furthermore, inhibition of Nodal function in aggressive melanoma cells either with a functional blocking antibody (49) or via Notch4 inhibition (50) reduces their VM-engaging ability.

In summary, our work underscores the importance of understanding the relationship between the unique tumor microenvironment and CSCs themselves. This is especially critical in light of the growing awareness of CSC plasticity and the challenge of therapeutically targeting cells based solely on surface markers. Future work should focus on the specific signaling pathways responsible for the ability of the CD133+/ABCB5+ subset to generate VM-dependent vascular niche, and the presumed reciprocal ability of the VM/vascular niche to induce the CD133+ phenotype from CD133− cells. Such studies will be essential for uncovering novel therapeutic targets and may facilitate a 2-pronged approach to cancer treatment that involves targeting stem cells as well as the signaling pathways responsible for inducing/sustaining them.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: C.-Y. Lai, M.-Y. Hsu
Development of methodology: C.-Y. Lai, M.-Y. Hsu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.-Y. Lai, M.-Y. Hsu
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.-Y. Lai, M.-Y. Hsu
Study supervision: M.-Y. Hsu

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CD133+ Melanoma Subpopulations Contribute to Perivascular Niche Morphogenesis and Tumorigenicity through Vasculogenic Mimicry

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