Tumor and Stem Cell Biology

VEGF-Mediated Angiogenesis Links EMT-Induced Cancer Stemness to Tumor Initiation

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Abstract

An epithelial–mesenchymal transition (EMT) underlies malignant tumor progression and metastatic spread by enabling cancer cells to depart from the primary tumor, invade surrounding tissue, and disseminate to distant organs. EMT also enriches for cancer stem cells (CSC) and increases the capacity of cancer cells to initiate and propagate tumors upon transplantation into immune-deficient mice, a major hallmark of CSCs. However, the molecular mechanisms promoting the tumorigenicity of cancer cells undergoing an EMT and of CSCs have remained widely elusive. We here report that EMT confers efficient tumorigenicity to murine breast cancer cells by the upregulated expression of the proangiogenic factor VEGF-A and by increased tumor angiogenesis. On the basis of these data, we propose a novel interpretation of the features of CSCs with EMT-induced, VEGF-A-mediated angiogenesis as the connecting mechanism between cancer cell stemness and tumor initiation. Cancer Res; 74(5); 1566–75. ©2014 AACR.

Introduction

A key feature of progressive solid tumors is the acquisition of the potential to invade neighboring tissue and to disseminate throughout the body and form metastatic lesions at distant sites (1–3). Tumor cells achieve this by activating an epithelial–mesenchymal transition (EMT) program to undergo phenotypic changes, such as the loss of cell–cell adhesion and the gain of migration capabilities to evade from the primary tumor. The tissue remodeling processes occurring during EMT are shared by embryonic development, wound healing, and metastasis formation. Molecular hallmarks of EMT are the loss of cell polarity, the loss of epithelial markers, such as E-cadherin and ZO-1, the gain of expression of mesenchymal markers, such as N-cadherin, vimentin, and fibronectin, a dramatic cytoskeletal reorganization accompanied by the change from an epithelial, differentiated morphology to a fibroblast-like, motile, and invasive cell behavior (3–5). Among many growth factors, TGF-β is one of the most potent inducers of EMT.

Interestingly, besides promoting invasiveness, TGF-β–induced EMT has been shown to induce the transition of transformed and immortalized human mammary epithelial cells into mesenchymal cancer cells with stem cell traits, thus linking EMT to tumor cell plasticity. In fact, EMT enriches for cancer stem cells (CSC) and increases tumorigenic potential of cancer cells upon transplantation into immunodeficient mice (6, 7). The ultimate hallmark of CSCs and metastatic cells is their ability to initiate tumors de novo (8). CSCs promote tumor growth through their ability to self-renew and to differentiate. However, the molecular mechanisms promoting the tumorigenicity of cancer cells undergoing EMT have remained widely elusive. Here, we report that EMT confers increased tumorigenicity to murine breast cancer cells by the upregulated expression of VEGF-A and by increased tumor angiogenesis. Notably, VEGF-A expression is required for the increased tumor initiation capacity of breast cancer cells that have undergone EMT. However, VEGF-A by itself is not sufficient to fully support tumor formation of epithelial breast cancer cells before EMT, indicating that additional factors induced during EMT contribute to efficient tumor initiation. We propose a novel interpretation of the features of CSCs with EMT-induced, VEGF-A-mediated angiogenesis as the connecting mechanism between cancer cell stemness and tumor initiation.

Materials and Methods

For details, see Supplementary Data.

Cell lines and reagents

A subclone of normal murine mammary gland epithelial (NMuMG) cells (NMuMG/E9; hereafter NMuMG) expressing E-cadherin has been described earlier (26). MTT/ECad and
MTΔECad cells have been previously described (9). Py2T cells were established from an MMTV-PyMT mammary gland tumor (10). All these cells have been derived or propagated in house for years and tested for mouse epithelial marker expression by reverse transcriptase (RT)-PCR and immunofluorescence stainings. MDA-MB-231 cells were a kind gift of N. Hynes (Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland) and were tested for human breast cancer markers by RT-PCR and immunofluorescence stainings. All cells were cultured in Dulbecco’s Modified Eagle Medium supplemented with glutamine, penicillin, streptomycin, and 10% fetal calf serum (Sigma-Aldrich).

**Orthotopic transplantation**

Two-dimensional (2D) cultured cells or second passage mammospheres (M2) were injected as a single-cell suspension in PBS at defined cell numbers into the ninth mammary gland of 7 to 10-week-old females BALB/c Rag2−/−:common γ receptor−/− (RG mice; a kind gift from A. Rolink, University of Basel, Basel, Switzerland).

**Lung metastasis and trap assay**

MMTV-Neu mice were injected intravenously with 106 MTIECad and MTΔECad cells resuspended in PBS. Three weeks or 3 days after injections, respectively, mice were sacrificed and lung metastases or GFP-positive cancer cells trapped in the lung were scored.

All experimental procedures involving mice were performed according to the guidelines of the Swiss Federal Veterinary Office (SFVO) and the regulations of the Cantonal Veterinary Office of Basel Stadt (licences 1878, 1907, 1908).

**Immunofluorescence microscopy**

Cryostat and paraffin sections were prepared as described previously (9). Seven micrometer cryostat sections of tumor samples were permeabilized with 0.1% Triton-X100 PBS, blocked with 5% goat serum or bovine serum albumin for 1 hour at room temperature, stained overnight at 4°C with primary antibodies against CD31 (1:50; 404274, BD Pharpingen), NG2 (1:100, AB5320 Millipore) followed by fluorescent secondary antibodies (Alexa Fluor; Invitrogen). Nuclei were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI). To assess tissue hypoxia, mice were injected intraperitoneally with 60 μg/kg hypoxpyroxa for 30 minutes before sacrifice and cryostat sections were processed according to the protocol (Hypoxpyroxa-1 Kit, HP1). Immunofluorescence pictures were acquired with a Leica DMI-4000. ImageJ was used for processing and analysis of the signal intensity.

Mammospheres were fixed in 4% paraformaldehyde, permeabilized with 0.1% Triton X-100 and stained overnight with antibodies against N-cadherin (33-3900 Zymed) and E-cadherin (13-1900 Zymed). Py2T cells were incubated with 5(6)-carboxyfluorescein N-hydroxysuccinimidyl ester (CFSE; 0.1 μmol/L) before spheroid culturing and unfixed spheroids were stained for living and dead cells, and with CFSE (green) to visualize label-retaining cells. Hoechst dye (blue) to detect cell nuclei, with propidium iodide (PI, red) to detect dead cells, and with CFSE (green) to visualize label-retaining cells. Scale bars, 100 μm. B, confocal microscopy analysis of epithelial MTIECad and mesenchymal MTΔECad cells grown for two passages under spheroid growth conditions. Cells were stained with Hoechst dye (blue) to detect cell nuclei, with propidium iodide (PI, red) to detect dead cells, and with CFSE (green) to visualize label-retaining cells. Scale bars, 100 μm. C, 3D reconstruction of epithelial (ECad) or MTΔECad cells expressing GFP were plated in 1% methylcellulose-containing medium to monitor clonal sphere formation. Quantification and representative GFP fluorescence microscopy pictures of colonies formed during 4 days of culture are shown. Scale bar, 200 μm.

**Statistical analysis**

The statistical significance between the tumor onset of two groups of mice was either tested by the Mann–Whitney U test or Fisher exact test in cases in which mice did not develop any tumors. For all further animal experiments, statistical significance was determined by the Mann–Whitney U test if not
stated differently in the figure legend. To compare values acquired in cell culture experiments, statistical significance was assessed by the Student t test. For all calculations, GraphPad Prism software was used.

Results

We employed defined in vitro EMT models of murine mammary epithelial cells and breast cancer cells to investigate the molecular mechanisms by which an EMT promotes the formation of CSCs and increases the tumorigenic potential of cancer cells. MTIECad cells have been established from a mammary tumor of an MMTV-Neu transgenic mouse carrying conditional (floxed) alleles of the E-cadherin gene. These cells undergo EMT upon Cre-mediated genetic ablation of the E-cadherin gene (MTDECad; ref. 9). NMuMG cells (9) and Py2T breast cancer cells, established from a mammary gland tumor of an MMTV-PyMT transgenic mouse (10), undergo EMT upon treatment with TGF-β. When cultured on antiadhesive plates, epithelial MTIECad, NMuMG, and Py2T cells formed unstructured cell aggregates, whereas the induction of EMT in these cells induced the growth of organized hollow spheres, a hallmark of stem cells (Fig. 1A–C and Supplementary Movies S1 and S2). The mesenchymal MTAECad hollow spheres expressed N-cadherin at cell–cell contacts, whereas the MTIECad epithelial cell aggregates employed E-cadherin for cell–cell adhesion (Fig. 1B). Culture in methylcellulose-containing media revealed significant clonal colony growth only with mesenchymal cells (Fig. 1D; ref. 11). EMT also provoked an increased sensitivity to the CSC-specific drug salinomycin (Supplementary Fig. S1; ref. 12). Consistent with the controversial debate on the use of cell surface markers for the identification of CSCs (13), the CSC surface markers CD24, CD29, and CD49f (14) failed to identify subpopulations of cells that were increased during EMT in the three murine cellular systems (Supplementary Table S1).

We next assessed the tumorigenic potential of epithelial MTIECad and mesenchymal MTAECad cells by orthotopic transplantation into the mammary fat pad of immunodeficient BALB/c Rag2+/−common γ receptor−/−(RG) mice. Notably, mesenchymal MTAECad cells initiated tumors faster and more efficiently than epithelial MTIECad cells. Limiting dilution experiments revealed that MTAECad cells exerted a significantly higher capacity of tumor initiation than MTIECad cells (Fig. 2A). When cells were cultured as spheroids before

Figure 2. Cells undergoing EMT exhibit a higher tumor initiation potential. A and B, MTIECad and MTAECad cells were cultured on plastic dishes (2D; A) or for two passages as spheroids (3D/M2; B) and transplanted in limiting dilutions into the ninth mammary fat pad of immunodeficient RG mice. The x-axis shows the cell numbers injected and the y-axis represents the time of first tumor detection in individual mice. The experiment was terminated 160 days after injection, and mice that had not developed tumors were recorded with 160 days. C, tumors formed by MTIECad and MTAECad cells in the mammary fat pad of RG mice were isolated and histologic sections were stained with hematoxylin and eosin. Light microscopy pictures show the tumor front (left) or the tumor center (right). Black squares indicate tumor areas that are shown as higher magnification in the top right corner. Scale bars, 100 μm and 10 μm in the magnified pictures. Statistical significance was calculated using the Fisher exact test. ***, P < 0.001; **, P < 0.01.
implantation (3D/M2), these differences became even more apparent (Fig. 2B). Upon spheroid selection, as few as 10 MT\textsuperscript{D}ECad cells were able to efficiently initiate a tumor, whereas 10 MT\textsuperscript{fl}ECad cells failed to do so. Epithelial MT\textsuperscript{fl}ECad cells gave rise to tumors with epithelial morphology, defined borders, and central necrotic areas, whereas tumors formed by MT\textsuperscript{D}ECad cells showed an invasive, fibroblast-like appearance in the absence of necrosis (Fig. 2C). Consistent with their increased tumorigenicity, mesenchymal MT\textsuperscript{D}ECad cells also formed more and larger lung metastases than epithelial MT\textsuperscript{fl}ECad cells following orthotopic transplantation into RG mice (Fig. 3A–C). Upon tail vein injection into syngeneic MMTV-Neu transgenic mice, epithelial MT\textsuperscript{fl}ECad cells seeded many small lung metastases, whereas mesenchymal MT\textsuperscript{D}ECad cells initiated less but larger lung metastases (Fig. 3D and E). No difference was observed in the ability of the cells to home to the lungs upon injection into the tail vein (Supplementary Fig. S2A), suggesting that the difference in metastatic outgrowth was due to tumor growth parameters at the distant site.

Both primary and metastatic MT\textsuperscript{D}ECad tumors exhibited significantly higher microvessel densities and reduced levels of apoptosis yet unchanged proliferation as compared with MT\textsuperscript{fl}ECad tumors and metastases (Fig. 4A–D and Supplementary Fig. S2B and S2C). MT\textsuperscript{D}ECad tumors also displayed higher levels of VEGF receptor 2 and 3 expression in their vasculature as compared with MT\textsuperscript{fl}ECad tumors (Supplementary Fig. S3A), indicating an activation of tumor angiogenesis. Lectin perfusion and pericyte coverage experiments showed that the vessels within the tumors and metastases were all functional.
and that MT\AECad tumors displays more perfused vessels than the MT\IECad ones (Supplementary Fig. S3B–S3D). In contrast to mesenchymal MT\AECad tumors, epithelial MT\IECad tumors also exhibited large hypoxic and necrotic areas associated with high levels of apoptosis yet unchanged proliferation (Fig. 4E and Supplementary Fig. S3E and S3F). Consistent with these findings, in a transgenic mouse model of pancreatic \beta-cell carcinogenesis (Rip1Tag2, RT2; ref. 15) loss of E-cadherin in the tumor cells (RT2;\beta-\AECad) not only correlated with increased tumor invasion and metastasis but also with intensified tumor angiogenesis (Fig. 4F and data not shown). Conversely, the genetic depletion of the transcriptional repressor of E-cadherin expression Snail-1 in Rip1Tag2 mice (RT2;\beta-\Snai1) resulted in reduced tumor invasion and tumor microvessel densities (Fig. 4F and data not shown). These results indicate a strong correlation between EMT and tumor angiogenesis. Gene expression profiling of MT\IECad, Py2T, and NMuMG cells before and after EMT revealed that, in addition to the activation of genes involved in cellular differentiation, cell motility, and adhesion, EMT also induced the expression of genes involved in the regulation of angiogenesis, including the gene encoding the proangiogenic factor VEGF-A (Supplementary Fig. S4A and S4B). A transient increase of VEGF-A levels by TGF-\beta has been previously reported in the absence of an EMT (16), yet in our in vitro models, increased VEGF-A expression is associated with the induction of an EMT.

The increased angiogenesis observed in tumors formed by MT\AECad cells correlated with high VEGF-A protein and mRNA levels in cultured MT\AECad cells (Fig. 5A and B). Spheroid culturing caused a further increase in VEGF-A levels (Fig. 5B) Notably, cultured MT\IECad cells expressed low amounts of VEGF-A compared with the MT\AECad cells. In contrast, high VEGF-A protein levels were found in large MT\AECad tumors (Fig. 5C), most likely due to the tumor hypoxia and necrosis observed in these tumors (Fig. 2C). We conclude that MT\AECad cells exhibit high VEGF-A levels already when implanted into mice, while MT\IECad with low levels of VEGF-A expression cells need to undergo an angiogenic switch for efficient tumor outgrowth. Supporting this notion, MT\AECad tumors smaller than 2 mm in diameter were highly vascularized, whereas small MT\IECad tumors widely lacked intratumoral microvessels (Fig. 5D).

To assess the functional contribution of VEGF-A to tumor initiation, we generated MT\IECad and MT\AECad cells that stably expressed shRNA against VEGF-A (shVA) or a nontargeting control shRNA (shCtr; Fig. 6A and Supplementary Fig. S5A). Upon EMT, silencing of VEGF-A significantly impaired tumor onset: 200 MT\AECad shCtr cells gave rise to tumors after 30 days, whereas no tumors were palpable at this time in mice transplanted with 200 MT\AECad shVA#1 or MT\IECad shCtr cells (Fig. 6B). Depletion of VEGF-A in epithelial MT\IECad shVA#1 cells only moderately delayed tumor onset as compared with MT\IECad shCtr cells. Independent experiments employing additional shRNA sequences against VEGF-A confirmed the reliance of early tumor onset on VEGF-A expression (Fig. 6C and D and Supplementary Fig. S5B). Consistent with their reduced expression of VEGF-A, MT\AECad shVEGF-A tumors exhibited significantly reduced...
microvessel densities as compared with MT\AEcad shCtr tumors; the levels of neovascularization were comparable with epithelial MT\AEcad tumors (Fig. 6E–G). Orthotopic implantation of 10 spheroid-cultured, VEGF-A–depleted MT\AEcad cells (shVA #1, #4, #5, #8) failed to provoke the efficient tumor formation observed with 10 MT\AEcad shCtr cells, further underscoring the critical requirement of VEGF-A for the increased tumor initiation of the cells after an EMT (Fig. 6H). Depletion of VEGF-A did neither significantly affect MT\AEcad and MT\AEcad cell proliferation nor mammosphere formation (Supplementary Fig. S5C–S5E), indicating that VEGF-A did not directly affect tumor cell proliferation and survival or cancer stemness. Finally, treatment of mice transplanted with MT\AEcad or MT\AEcad cells with the VEGF receptor inhibitor PTK787/ZK222584 (PTK) significantly impaired vascularization and growth of both tumor types (Fig. 7A and B), indicating that VEGF-A upregulation plays a role only in the context of an EMT-induced mechanism. These results indicate that, while critical for tumor angiogenesis, VEGF-A by itself is not sufficient to efficiently promote tumor initiation. Besides VEGF-A, the other family members VEGF-B, C, and D were also found upregulated in their expression during EMT and in spheroid culture of MT\AEcad and MT\AEcad cells and tumors, yet to varying degrees and without affecting tumor lymphangiogenesis (Supplementary Fig. S7A–S7D). Moreover, shRNA-mediated ablation of VEGF-C expression failed to affect MT\AEcad tumor formation (Supplementary Fig. S7E and S7F). Hence, other factors were likely necessary to support VEGF-A in the tumorigenic abilities of mesenchymal MT\AEcad cells. High levels of matrix metalloproteinases (MMP), inflammatory cytokines, and activated endothelial cell markers were found in lysates of MT\AEcad tumors as
compared with MT\textsubscript{fl}ECad tumors (Supplementary Table S2), suggesting that additional proangiogenic signaling pathways contributed to the efficient tumor initiation of MT\textsubscript{Δ}ECad cells. Consistent with this notion, conditioned medium of MT\textsubscript{Δ}ECad cells supported the growth of human umbilical vein endothelial cells (HUVEC), while MT\textsubscript{fl}ECad-conditioned media did not (Supplementary Fig. S8). Accordingly, forced expression of VEGF-A in MT\textsubscript{fl}ECad cells increased, whereas VEGF-A knockdown in MT\textsubscript{Δ}ECad cells decreased conditioned medium-induced HUVEC proliferation.

Figure 6. Tumor cell–derived VEGF-A is required for early tumor onset. A, secreted VEGF-A protein levels were assessed by ELISA in the supernatant of MT\textsubscript{fl}ECad and MT\textsubscript{Δ}ECad cells that have been infected to express either a nontargeting control shRNA (shCtr) or an shRNA against VEGF-A (shVA #1). The means of three independent measurements are plotted with SEM. Statistical significances were calculated using a paired Student t test. *, P < 0.05. B, two hundred MT\textsubscript{fl}ECad and MT\textsubscript{Δ}ECad cells stably expressing either shRNA against VEGF-A (shVA #1) or control shRNA (shCtr) were injected into the ninth mammary fat pad of RG mice. Tumor formation was monitored by palpation. Statistical significance was evaluated by the Mann–Whitney U test. ***, P < 0.01. C and D, additional small hairpins against VEGF-A (shVA #4, shVA #5, shVA #8) were used to validate the result with shVA #1. Knockdown efficiency was tested by measuring the levels of VEGF-A in the cell supernatants by ELISA. The mean of three independent measurements is plotted ± SEM (C). Tumor onset (D) was determined as described in B. E, vascularization of MT\textsubscript{Δ}ECad shCtr and shVA #1 tumors was analyzed by immunofluorescence staining for CD31 (green). Representative immunofluorescence microscopy pictures are shown. Scale bars, 100 μm. F and G, the degree of vascularization was quantified by counting the number of CD31-positive vessels per area (F) and the area fraction of CD31-positive vessels (G). Statistical analysis of the quantifications was performed using a Mann–Whitney U test. ***, P < 0.001; N = 4 mice. H, MT\textsubscript{Δ}ECad expressing a control shRNA (shCtr) or shRNAs targeting VEGF-A (shVA #1, #4, #5, #8) was cultured for two passages as spheroids (M2). After dissociation of the spheroids, 10 cells in PBS were injected into the ninth fat pad of RG mice. Tumor onset was monitored and plotted. The experiment was finally terminated 160 days after injection and mice that had not developed tumors were recorded with 160 days. Statistical analysis of the quantifications was performed using the Fisher exact test. ***, P < 0.005; ***, P < 0.001.
To investigate the possibility that VEGF-A upregulation could have implications on the CSC's intrinsic properties, we analyzed the VEGFR1 and Nrps levels in MT\textsubscript{fl}ECad and MT\textsubscript{D}ECad in 2D or spheroids culturing, as well the effect of downregulation of Nrp1 and Nrp2 on cell proliferation and cell cycle. In contrast to what has been previously shown in other models (17), we did not observe any effect of VEGFR and Nrp expression on cell proliferation or mammosphere formation in our cellular models (data not shown).

To determine VEGF-A expression by tumorigenic CSCs of human breast tumors, we isolated \textit{bona fide} CSCs from patients' primary breast tumors that have been previously serially transplanted in immunocompromised mice (18) as well as from the human breast cancer cell line MDA-MB-231. Single-cell suspensions were stained with antibodies against CD44 and CD24. CD44\textsuperscript{+}/CD24\textsuperscript{low}/C0\textsuperscript{low} putative CSC and CD44\textsuperscript{+}/CD24\textsuperscript{low} non-CSCs (19) were isolated by flow cytometry, and the expression of VEGF-A mRNA was determined by qRT-PCR. Indeed, the putative CSC population exhibited significantly higher levels of VEGF-A mRNA expression than their non-CSC counterparts (Fig. 7C and D), indicating that also in human breast cancer cells, increased cancer cell stemness correlated with high VEGF-A expression.

**Discussion**

In this report, we have utilized standardized assays for CSCs to delineate the mechanisms underlying EMT-induced cancer stemness. While spheroid growth, tumor initiation, and metastatic spread revealed a correlation between EMT and hallmarks of CSCs (shown here), the analysis of cell surface markers (Supplementary Table S1), aldehyde dehydrogenase-positive populations, label-retaining cells, and general drug resistance (data not shown) did not correlate with EMT. While the identification of appropriate stem cell markers for the isolation of CSCs remains challenging (13), growing evidence links tumor-initiating cells with proangiogenic signals (17, 20, 21). Notably, we have identified VEGF-A–mediated angiogenesis as one critical determinant of the increased tumorigenicity of cells undergoing EMT: (i) VEGF-A and several other angiogenic factors and cytokines are upregulated in murine breast cancer cells during EMT, (ii) CSCs of human primary breast cancers or from a human breast cancer cell line also display increased levels of VEGF-A, (iii) VEGF-A is required for the increased tumorigenicity of cells undergoing EMT, yet (iv) VEGF by itself is not sufficient to promote tumorigenicity of epithelial cancer cells, indicating that additional angiogenic factors regulated in their expression during EMT are critical for an effective tumor initiation. Our results also show that VEGF-A is required for EMT-induced angiogenesis in the early events of tumor initiation, in line with similar results in skin and brain (17) and with an established central role for VEGF-A in tumor progression (22–24).

As few as 10 mesenchymal cells efficiently initiated tumors upon orthotopic transplantation, while their epithelial counterparts were much less efficient. The calculated CSC frequency of MT\textsubscript{D}ECad [1:14.3 and 1:23.3 with cells cultured as
mammospheres (3D/M2) and on plastic (2D), respectively) and of MTBEcad cells (1: 398 and 1:73.7 with 3D/M2 and 2D cells, respectively could not be statistically verified, since extreme limiting dilution analysis (25) rejected a single hit model for MTAEcad cells, thus arguing in favor of a multi-hit event (LR test $P = 0.073$ for 3D/M2 and $P = 0.0065$ for 2D in MTAEcad cells, and $P = 0.178$ for 3D/M2 and $P = 0.193$ for 2D in MTIEcad). These results are consistent with the observation that VEGF-A by itself is not sufficient to increase the tumorigenicity of epithelial cancer cells and that other factors are required as well. Together, these findings propose a novel interpretation of cancer stemness by functionally linking EMT with VEGF-A-mediated tumor angiogenesis and the capacity to initiate tumors de novo. These results provide important insights into the mechanisms underlying the increased tumorigenicity of CSCs and cells undergoing EMT and open avenues for the design of therapeutic interventions. Our work also challenges the hypothesis that the ability of CSCs to de novo initiate tumors is exclusively due to features specifically attributed to CSCs, such as asymmetric cell division and self-renewal. Rather, a high angiogenic potential, for example, induced by an EMT and the upregulated expression of VEGF-A, contributes to the tumorigenic phenotype of CSCs. Our data indicate that high angiogenic capabilities are an intrinsic feature of CSCs and metastatic cells. The data also raise the caveat that, while tumorigenicity is a hallmark of metastatic cells and CSCs (tumor-initiating cells), tumorigenicity assays may not be the ultimate criteria to definitively identify CSCs.

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

References


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doi:10.1158/0008-5472.CAN-13-1641

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