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Tumor Initiation in Human Malignant Melanoma and Potential Cancer Therapies

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Abstract

Cancer stem cells (CSCs), also known as tumor-initiating cells, have been identified in several human malignancies, including human malignant melanoma. The frequency of malignant melanoma-initiating cells (MMICs), which are identified by their expression of ATP-binding cassette (ABC) family member ABCB5, correlates with disease progression in human patients. Furthermore, targeted MMIC ablation through ABCB5 inhibits tumor initiation and growth in preclinical xenotransplantation models, pointing to potential therapeutic promise of the CSC concept. Recent advances also show that CSCs can exert pro-angiogenic roles in tumor growth and serve immunomodulatory functions related to the evasion of host anti-tumor immunity. Thus, MMICs might initiate and sustain tumorigenic growth not only as a result of CSC-intrinsic self-renewal, differentiation and proliferative capacity, but also based on pro-tumorigenic interactions with the host environment.

Keywords

Cancer stem cells; Melanoma; Malignant melanoma-initiating cells; Melanoma stem cells; ABCB5; Angiogenesis; Immunity

INTRODUCTION

While overall cancer incidence rates have decreased in the United States, the incidence rate of human malignant melanoma continues to increase [1]. As the most malignant skin cancer, melanoma is the sixth most common cancer in men and the seventh in women in the United States [1]. When diagnosed before the cancer has spread, melanoma can be cured by surgical resection [2]. However, metastatic melanoma has a poor prognosis and is one of the most aggressive and drug-resistant cancers, with a median survival time of 6 months and a 5-year survival rate of less than 5% [2]. Currently, there is no effective therapy available that can significantly alter the course of patients with metastatic melanoma. Hence, there exists a profound need for the development of new therapies for metastatic melanoma. In this regard, cancer stem cells (CSCs) might represent a promising novel target for melanoma therapy.

According to the CSC theory [3], tumors can be composed of heterogeneous cancer cell subpopulations, of which only CSCs can initiate and perpetuate tumor growth (Fig. 1). At the same time, CSCs give rise, through differentiation, to non-CSC subpopulations that are no longer capable of driving tumor growth. To date, CSCs have been identified in several human

malignancies [4,5], including in human malignant melanoma [6]. Given the important roles of CSCs in the initiation and progression of human malignancies [4,5], it will likely be necessary to target and eliminate these aggressive cancer subpopulations, in addition to the killing of cancer bulk populations, in order to achieve patient cures, including in melanoma (Fig. 2). Importantly, proof-of-principle for the potential therapeutic utility of the CSC concept has recently been established by demonstrating that selective killing of MMICs through their prospective marker, ABCB5, can inhibit tumor growth [6]. This proof-of-principle has since been extended to additional malignancies that harbor CSCs [4,5], indicating a broader therapeutic promise of the CSC concept.

Here, we discuss the heterogeneity of human melanomas for cells of varying tumorigenic potential, and the identification of CSCs in human malignant melanoma, i.e. MMICs. Moreover, we review recent research advances regarding pro-tumorigenic interactions of CSCs with the tumor host environment, which include pro-angiogenic functions and MMIC-mediated modulation of host anti-tumor immunity.

MALIGNANT MELANOMA AND CANCER STEM CELLS

Melanoma Heterogeneity for Cells of Varying Tumorigenic Potential

Several studies have identified melanoma heterogeneity for malignant cells of varying tumorigenic potential. These studies pointed to the existence of MMICs, because enhanced tumorigenicity represents one cardinal feature of CSCs [7]. However, it should be noted that demonstration of tumorigenic potential alone is not sufficient as a criterion to attribute CSC phenotype and function, unless prolonged self-renewal and differentiation capacity of a defined cancer cell subset is also demonstrated in serial *in vivo* xenotransplantation experiments [4,5, 7].

Fang *et al.* generated melanoma spheres by culturing human melanoma cells in mouse embryonic fibroblast-conditioned human embryonic stem cell medium [8]. When xenografted subcutaneously to severe combined immune deficient (SCID) mice, sphere-derived melanoma cells showed a moderate increase in relative tumorigenicity compared to melanoma cells derived from adherent cultures (3/5 vs. 1/5 tumors, respectively) [8].

Grichnik *et al.* found melanomas to be heterogeneous for the Hoechst dye side population (SP) phenotype [9]. The SP phenotype was first identified in bone marrow hematopoietic stem cells [10] and is thought to arise from enhanced cellular dye efflux as a result of expression of ATP-binding cassette (ABC) transporter activity, a class of molecules involved in cellular xenobiotic efflux transport and cancer multidrug resistance (MDR) [11]. Grichnik *et al.* showed that despite lower *in vitro* proliferation rates, melanoma SP cells exhibited more efficient tumorigenic growth *in vivo* [9].

However, neither melanoma spheres [8] nor melanoma SP cells [9] have been defined by a molecular marker or set of markers. This might impede efforts to detect these cell populations *in situ* in human melanoma tissue, to further define the molecular mechanisms underlying their tumor-initiating capacity, and to identify cellular targets in these distinct melanoma subpopulations that might be suitable candidates for therapeutic drug development.

The CD133 Marker and Potential Targeted Therapies

Regarding heterogeneously expressed molecular markers, our laboratory identified CD133, a transmembrane glycoprotein of unknown physiological function, to be expressed on subpopulations of cultured human epidermal melanocytes (HEM) [12] and human melanomas [13]. In a larger series of clinical specimens, Klein *et al.* subsequently also detected heterogeneous CD133 expression in melanocytic tumors, in 17% of benign nevi, 39% of

primary melanomas, and 46% of metastatic melanomas using a tissue microarray of 226 melanocytic lesions [14]. Monzani *et al.* investigated the role of CD133 as a cell surface marker for tumorigenic melanoma cells [15]. Xenotransplantation of human biopsy-derived melanoma cells to nonobese diabetic (NOD)/SCID mice showed that only CD133⁺ cells, which represented 0.2% – 0.8% of the overall melanoma cell population, induced subcutaneous tumor formation [15]. In contrast, Quintana *et al.* found no enrichment of tumorigenic potential among CD133⁺ *versus* to CD133[−] melanoma cells upon xenotransplantation to interleukin-2 receptor gamma (IL2Rgamma)^{−/−} NOD/SCID mice in the presence of extracellular matrix supplementation [16]. These data indicate a possible dependence of tumorigenic phenotype on tumor host characteristics [4,5] or that, alternatively, CD133 might not represent a universal marker for increased tumorigenic potential in all human melanomas.

Importantly, the role of CD133 as a potential therapeutic target for melanoma therapy has also been investigated [17]. Rappa *et al.* found dose-dependent inhibition of cell proliferation upon treatment of cultured human FEMX-1 melanoma cells with CD133 antibody [17]. Moreover, short hairpin RNA (shRNA) knockdown of CD133 expression in human melanoma cells reduced proliferation and cellular migration *in vitro*, and inhibited the development of pulmonary and spinal cord metastases following intravenous tumor cell inoculation *in vivo* [17].

The Nodal Marker and Potential Targeted Therapies

Nodal, a member of the TGF- β family, is involved in stem cell maintenance and differentiation [18]. Expression of Nodal correlates positively with melanoma progression [18]. For example, Nodal protein was not detected in normal skin, was absent or weakly expressed in primary melanoma, but could be readily detected in 45–60% of cutaneous melanoma metastases [18]. Of therapeutic interest, inhibition of Nodal expression by antisense oligonucleotides suppressed colony formation activity of melanoma cells *in vitro* and inhibited primary tumor growth *in vivo* [18]. In addition, blockade of the Nodal signalling pathway through the Nodal inhibitor Lefty, anti-Nodal morpholino, or small molecule ALK receptor inhibitors induced differentiation of Nodal-positive metastatic melanoma cells and suppressed their tumorigenic potential [19].

In aggregate, there exists evidence for melanoma heterogeneity for marker-defined subpopulations of varying tumorigenic potential. While these findings might depend in certain instances on the particular melanoma specimens studied or on the characteristics of the experimental tumor host employed, they have pointed to the existence of CSCs in human malignant melanoma.

Identification of Malignant Melanoma-Initiating Cells

The CSC theory postulates that cancers can be hierarchically organized, and that CSCs found at the apex of this cellular hierarchy comprise only a subpopulation of tumor cells that is essential for its propagation [3]. CSCs may be operationally defined as a human cancer biopsy-derived tumor subpopulation, prospectively identifiable by a molecular marker or marker combination, which, unlike cancer bulk populations negative for the particular marker or marker combination, can initiate tumor formation and growth in serial xenotransplantation experiments in immunocompromised mice *in vivo*. Furthermore, CSCs are thought to possess the exclusive capacity for self-renewal and for giving rise to non-tumorigenic bulk populations of cancer cells, thereby recapitulating clinical parent tumor heterogeneity [3]. The CSC model does not make any specific assumptions about the relative frequency of CSCs or about multipotent differentiation plasticity of CSC subsets [4,5,7]. Moreover, the CSC definition makes no specific assumptions about the malignant cell-of-origin, which can be represented

by a physiological stem cells but also by committed progenitor cells and even terminally differentiated cells [4,5,7].

ABCB5-Expressing MMICs and Targeted Therapies

We recently identified a subpopulation of CSCs in human malignant melanoma, termed MMICs [6], based on expression of the MDR transporter ABCB5 (ABC, sub-family B (MDR/TAP), member 5) [12]. ABCB5 is expressed by physiological HEM progenitors [12] as well as by primary and metastatic human melanomas [6,13] and established melanoma cell lines [12,13,20]. In clinical melanoma specimens, ABCB5⁺ tumor cells ranged in frequency from 2 to 20% [6]. Xenotransplantation of patient-derived ABCB5⁺ human melanoma cells across a log-fold range to primary NOD/SCID murine recipients resulted in tumor formation in 14 of 23 experimental animals compared to tumor formation in only 1 of 23 primary recipients of ABCB5⁻ tumor xenografts [6]. Importantly, only ABCB5⁺ melanoma cells but not ABCB5⁻ melanoma bulk populations induced tumor formation in secondary xenograft recipients. Furthermore, histological examination of ABCB5⁺ melanoma cell-derived primary and secondary xenograft tumors revealed phenotypic heterogeneity resembling that of the respective parent tumors [6]. The identified association of ABCB5 expression with tumorigenesis is consistent with findings of close co-regulation of ABCB5 with melanoma tumor antigen p97 (melanotransferrin (MTf)), a known regulator of tumor growth [21] and with preferential ABCB5 expression also by *in vitro* clonogenic, self-renewing melanoma cell subsets [22]. Furthermore, it is consistent with the downregulation of ABCB5 with induced terminal differentiation and the concomitant loss of growth potential and chemoresistance of human melanoma cells [23].

In order to examine the relative contribution of co-xenografted ABCB5⁺ and ABCB5⁻ subpopulations to tumor growth, self-renewal, and differentiation *in vivo*, we performed genetic lineage tracking of sorted ABCB5⁺ and ABCB5⁻ tumor cells using DsRed (red fluorescent protein) and EYFP (enhanced yellow-green fluorescent protein) fluorescent genetic markers, respectively [6]. Xenotransplantation to NOD/SCID mice of ABCB5⁺/DsRed⁺ melanoma subsets and ABCB5⁻/EYFP⁺ tumor bulk components reconstituted at the naturally occurring relative abundance of approximately 1:10 resulted in markedly increased relative frequencies (up to a frequency of 50%) of DsRed⁺ cells of ABCB5⁺ origin in tumor xenografts at the experimental endpoint of six weeks [6]. These results showed an enhanced tumorigenicity of ABCB5⁺ subsets in a competitive tumor development model [6]. ABCB5⁺ melanoma cells re-isolated from experimental tumors were exclusively of red fluorescent phenotype (ABCB5⁺ origin), thereby demonstrating self-renewal capacity of this cell subset, whereas EYFP⁺ cells (ABCB5⁻ origin) were not found at significant levels among ABCB5⁺ isolates [6]. These findings demonstrated that ABCB5⁺ tumor cells arose only from ABCB5⁺ inocula, and that ABCB5⁻ cells gave rise exclusively to ABCB5⁻ progeny. Fluorescent ABCB5⁻ tumor cell isolates exhibited both DsRed positivity and EYFP positivity, demonstrating that ABCB5⁺ cells possess the capacity to differentiate into ABCB5⁻ cell subpopulations [6]. These findings provided initial *in vivo* evidence for a hierarchical tumor organization in human malignant melanoma as posited by the CSC theory, with tumorigenic ABCB5⁺ cancer cells enriched for CSCs possessing the exclusive capacity to self-renew and to give rise to more differentiated, ABCB5⁻ tumor progeny [6].

Importantly, abundance of the CSC subset identified by ABCB5 correlates positively with neoplastic progression in human melanoma patients [6]. Quantification of ABCB5 staining intensity of an established melanocytic tumor progression tissue microarray revealed that primary or metastatic melanomas expressed significantly more ABCB5 than benign melanocytic nevi, thick primary melanomas more than thin primary melanomas, and melanomas metastatic to lymph nodes more than primary lesions [6]. Consistent with these

findings, the ABCB5 gene is also preferentially expressed by melanomas with high *in vivo* tumorigenic capacity in human to murine xenotransplantation models [24,25] and by melanomas of metastatic as opposed to primary tumor origin [26]. Thus, ABCB5 provides a direct and unique link between CSCs, cancer therapeutic resistance, and neoplastic progression in human malignant melanoma.

We therefore examined whether targeted ablation of a prospectively identified CSC compartment inhibits tumor growth [6]. ABCB5 monoclonal antibody (mAb) administration to nude mouse recipients of human melanoma xenografts significantly inhibited tumor growth compared to controls over the course of a 58-day observation period, through MMIC-specific antibody-dependent cell mediated cytotoxicity (ADCC) [6]. ABCB5 mAb treatment also significantly inhibited tumor formation, with tumors detected in only 3/11 ABCB5 mAb-treated mice *versus* 10/10 control antibody-treated mice and 18/18 untreated controls [6]. Upon cessation of mAb administration, only one tumor occurrence was noted among the eight ABCB5-treated mice that had not developed a tumor, during an additional eight-month observation period, indicating prolonged inhibition of MMICs [6]. ABCB5 mAb treatment of established human-to-nude mouse melanoma xenografts also significantly inhibited tumor growth compared to controls. Immunohistochemical analysis of ABCB5 mAb-treated patient-derived melanoma xenografts revealed only small, significantly reduced foci of ABCB5 expression (overall <1% of cells) with CD11b⁺ macrophage infiltration corresponding to regions of ABCB5 mAb localization that frequently bordered zones of cellular degeneration and necrosis, implicating ADCC as the mechanism underlying CSC depletion and inhibition of tumor growth. In contrast, control treated xenografts revealed 10–15% ABCB5-reactive cells, secondary anti immunoglobulin mAb failed to localize to the respective regions in adjacent sections, and CD11b⁺ macrophages failed to infiltrate the tumor tissue [6].

These findings provided initial proof-of-principle that targeted ablation of MMICs through a defining molecular marker is sufficient to significantly inhibit tumorigenesis and cancer growth, thereby validating the potential therapeutic utility of the CSC concept [6]. In this regard, ABCB5 represents a particularly promising MMIC therapeutic target, because ABCB5 mediates MDR in human melanoma [13,27,28]. For example, mAb- or small inhibitory RNA (siRNA)-mediated ABCB5 inhibition can sensitize melanoma cells to killing by doxorubicin [13,27], 5-fluorouracil (5-FU) or camptothecin [28]. ABCB5 targeting strategies are currently in clinical development for cancer therapy. In addition, CSC/tumor host interactions might represent additional targets for therapeutic intervention.

CSC Interactions with the Tumor Host Environment and Potential Cancer Therapies

The identification and molecular characterization of CSCs in human malignancies has allowed initiation of studies regarding their interactions with the tumor host environment. It is increasingly recognized that in order for a transformed, malignant cell to grow into a clinically significant tumor mass, multiple barriers imposed by the host organism need to be surpassed [4]. Therefore, the potential of a CSC to form cancer might not only be determined by its intrinsic self-renewal, differentiation and proliferative potential, but also by its ability to modulate and adapt to the environment of its host to favor tumor growth. In this regard, angiogenesis and vasculogenic mimicry, and host anti-tumor immunity show specific relationships to CSCs that significantly impact tumor growth (Fig. 1).

Functions of CSCs in Angiogenic Process and Vasculogenic Mimicry and Cancer Therapies

Angiogenesis is a hallmark of tumor development and the control of tumor angiogenesis is an integral part of the host defense response to tumor growth [29]. The shift of the tumor microenvironment to an angiogenic state, or ‘angiogenic switch’, is an important rate-limiting factor in tumor development [30]. However, the reciprocal relationship between CSCs and

tumor angiogenesis was not revealed until recently. Some studies have shown that the tumor vasculature and blood/lymphatic circulation can directly affect the status of CSCs. For instance, hypoxia can promote expansion of CD133⁺ brain CSCs in glioma *via* activation of hypoxia-inducible factor-1 alpha (HIF-1 α) expression [31]. A perivascular niche has been identified for brain CSCs, in which the numbers of endothelial cells and blood vessels directly impact CD133⁺ CSC proliferation and tumor growth [32]. CSCs can also actively modulate angiogenic activity in human tumors. When xenografted to immunodeficient mice, CD133⁺ brain CSCs were not only found to be more tumorigenic than their CD133⁻ counterparts, but also shown to generate tumors with higher microvessel density and increased vascular endothelial growth factor (VEGF) content [33,34]. Elevated VEGF expression in brain CSCs in glioma was found to be at least in part a result of up-regulated HIF-2 α expression and could still be detected under normoxia conditions, suggesting that it represents an intrinsic feature of glioma-initiating cells [35].

In addition to endothelial cells surrounding the tumor mass, bone marrow-derived cells have also been proposed to contribute to the development of the tumor vasculature [36]. Similar to CD133⁺ CSCs in glioma, glioma-initiating cells identified by sphere-forming capability also generated tumors with higher vascular density [37]. Moreover, tumors derived from these subpopulations mobilized and recruited endothelial progenitor cells from bone marrow more effectively [37]. It is conceivable that at early stages of tumor development, malignant growth is supported by preexisting blood vessels, which maintain a tumor microenvironment with physiological oxygen concentration. Thus, most tumor cells might not express pro-angiogenic factors at significant levels and therefore might not induce tumor angiogenesis. In contrast, intrinsically high VEGF expression of CSCs in gliomas [35], or vasculogenic mimicry [38] by MMICs in melanomas [6], might enable these tumorigenic subpopulations to induce angiogenesis or vasculogenic mimicry in the absence of hypoxia, which could provide important support for tumor initiation. Indeed, some human tumor xenografts can develop into microscopic lesions and remain at this stage over an extended period of time, despite the presence of proliferating cells within the tumor [39]. This might be explained by the limited expression of pro-angiogenic factors by the bulk of tumor cells within such lesions with potentially only a limited presence of CSCs, resulting in a lag time for the triggering of the angiogenic switch and thus the initiation of more robust tumor growth. As increasing numbers of tumor cells proliferate, the tumor mass might eventually outgrow the support capacity of the existing vasculature, resulting in decreased levels of oxygen and nutrients and increased accumulation of metabolic waste products. Responding to this deterioration of the tumor environment, not only CSCs, but also tumor bulk populations might up-regulate pro-angiogenic factor expression, enhancing angiogenesis and tumor growth.

Importantly, treatment with anti-VEGF mAb has been shown to significantly inhibit the growth of CD133⁺ CSC-derived xenografts, indicating that increased VEGF expression by glioma CSCs can be targeted therapeutically [33]. Also in human glioma xenografts, the number of sphere-forming units was significantly reduced when cytotoxic treatment was combined with antiangiogenic therapy, indicating that glioma CSCs can be chemosensitized by the disruption of their vascular niches [40]. As increasing numbers of anti-angiogenic agents reach different stages of drug development, the pro-angiogenic activity of CSCs might therefore provide a promising target for therapeutic intervention.

Immunomodulatory functions of MMICs and Cancer Therapies

In addition to pro-angiogenic functions, CSCs might induce tumorigenic growth through inhibition of host anti-tumor immune effector responses [41,42] (Fig. 1). Indeed, observations in experimental cancer models and human patients have pointed to a negative correlation between host immunocompetence and rates of tumor growth [41]. For example, while

estimated CSC frequencies in human melanoma xenotransplantation models involving NOD/SCID mice are low [6,16], higher frequencies of cells capable of initiating melanoma xenografts may be detected when utilizing more severely immunocompromised IL-2R $\gamma^{-/-}$ NOD/SCID hosts [16]. Moreover, even highly immunogenic tumors such as melanoma [43] are capable of inexorable tumor growth despite the presence of robust host immunity.

These findings have raised the possibility that under conditions of relatively intact immunity, only a restricted minority of malignant cells, i.e. CSCs, might possess the phenotypic and functional characteristics to evade host immunosurveillance and immune-mediated rejection [41]. Recently, our laboratory provided evidence supporting this hypothesis, by demonstrating that tumorigenic ABCB5⁺ MMICs possess the capacity to preferentially inhibit IL-2-dependent T cell activation [42]. This study also showed that ABCB5⁺ MMICs support, in a B7.2-dependent manner, regulatory T (Treg) cell induction [42]. We found that ABCB5⁺ MMICs, compared to melanoma bulk populations, expressed lower levels of the major histocompatibility complex (MHC) class I, showed aberrant positivity for MHC class II, and exhibited lower expression levels of the melanoma-associated antigens (MAAs), melanoma antigen recognized by T cells-1 (MART-1), melanoma inhibitor of apoptosis protein (ML-IAP), NY-ESO-1, and MAGE-A [42]. In addition, our results showed that tumorigenic ABCB5⁺ subpopulations preferentially expressed the costimulatory molecules B7.2 and programmed cell death-1 (PD-1) in both established melanoma xenografts and clinical tumor specimens *in vivo* [42]. In immune activation assays, ABCB5⁺ melanoma cells inhibited mitogen-dependent human peripheral blood mononuclear cell (PBMC) proliferation and IL-2 production more efficiently than ABCB5⁻ populations [42]. Moreover, coculture with ABCB5⁺ MMICs increased, in a B7.2 signalling-dependent manner, CD4⁺/CD25⁺/FoxP3⁺ Treg cell abundance and IL-10 production by mitogen-activated PBMCs [42]. Consistent with these findings, we found that ABCB5⁺ melanoma subsets also preferentially inhibited IL-2 production and induced IL-10 secretion by cocultured patient-derived, syngeneic PBMCs [42]. These findings identified novel T cell-modulatory functions of ABCB5⁺ melanoma subpopulations and point to specific roles for MMICs in the evasion of antitumor immunity and in cancer immunotherapeutic resistance.

The recognition of MMIC-dependent immunomodulation is highly relevant to human melanoma therapy. It provides the rationale to examine in clinical trials whether current approved or investigational immunotherapeutic strategies that employ IL-2 [44] or target the B7-CD28/CTLA-4 or PD-1 signalling pathways in melanoma patients [45,46] might function in part to inhibit ABCB5⁺ MMIC-induced tumor immune-evasion and immunologic tolerance.

CONCLUSION

CSCs have been identified in several human malignancies, including malignant melanoma. While CSCs often represent only a minority of all cancer cells in a tumor, they represent the dominant force driving tumor growth. In addition to intrinsic self-renewal, differentiation and proliferative capacity, CSCs can exert proangiogenic roles in tumor growth and, in human melanoma, serve immunomodulatory functions related to the evasion of host anti-tumor immunity. These protumorigenic interactions with host environmental factors likely contribute to their unique roles in tumor initiation and growth and represent additional targets for therapeutic intervention.

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REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics 2008. *CA Cancer J. Clin* 2008;58(2):71–96. [PubMed: 18287387]
2. Cummins DL, Cummins JM, Pantle H, Silverman MA, Leonard AL, Chanmugam A. Cutaneous malignant melanoma. *Mayo Clin. Proc* 2006;81(4):500–507. [PubMed: 16610570]
3. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001;414(6859):105–111. [PubMed: 11689955]
4. Frank NY, Schatton T, Frank MH. The therapeutic promise of the cancer stem cell concept. *J. Clin. Invest* 2010;120(1) in press.
5. Schatton T, Frank NY, Frank MH. Identification and targeting of cancer stem cells. *Bioessays* 2009;31(110):1038, 1049. [PubMed: 19708024]
6. Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, Zhan Q, Jordan S, Duncan LM, Weishaupt C, Fuhlbrigge RC, Kupper TS, Sayegh MH, Frank MH. Identification of cells initiating human melanomas. *Nature* 2008;451(7176):345–349. [PubMed: 18202660]
7. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, Visvader J, Weissman IL, Wahl GM. Cancer stem cells—perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res* 2006;66(19):9339–9344. [PubMed: 16990346]
8. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, Van Belle PA, Xu X, Elder DE, Herlyn M. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 2005;65(20):9328–9337. [PubMed: 16230395]
9. Grichnik JM, Burch JA, Schulteis RD, Shan S, Liu J, Darrow TL, Vervaert CE, Seigler HF. Melanoma, a tumor based on a mutant stem cell? *J. Invest. Dermatol* 2006;126(1):142–153. [PubMed: 16417230]
10. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *J. Exp. Med* 1996;183(4):1797–1806. [PubMed: 8666936]
11. Gottesman MM, Fojo T, Bates SE. *Nat. Rev. Cancer* 2002;2(1):48–58. [PubMed: 11902585]
12. Frank NY, Pendse SS, Lapchak PH, Margaryan A, Shlain D, Doeing C, Sayegh MH, Frank MH. Multidrug resistance in cancer: role of ATP-dependent transporters. *J. Biol. Chem* 2003;278(47):47156–47165. [PubMed: 12960149]
13. Frank NY, Margaryan A, Huang Y, Schatton T, Waaga-Gasser AM, Gasser M, Sayegh MH, Sadee W, Frank MH. ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma. *Cancer Res* 2005;65(10):4320–4333. [PubMed: 15899824]
14. Klein WM, Wu BP, Zhao S, Wu H, Klein-Szanto AJ, Tahan SR. Increased expression of stem cell markers in malignant melanoma. *Mod. Pathol* 2007;20(1):102–107. [PubMed: 17143262]
15. Monzani E, Facchetti F, Galmozzi E, Corsini E, Benetti A, Cavazzin C, Gritti A, Piccinini A, Porro D, Santinami M, Invernici G, Parati E, Alessandri G, La Porta CA. Melanoma contains CD133 and ABCG2 positive cells with enhanced tumourigenic potential. *Eur. J. Cancer* 2007;43(5):935–946. [PubMed: 17320377]
16. Quintana E, Shackleton M, Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. *Nature* 2008;456(7222):593–598. [PubMed: 19052619]
17. Rappa G, Fodstad O, Lorico A. The stem cell-associated antigen CD133 (Prominin-1) is a molecular therapeutic target for metastatic melanoma. *Stem Cells* 2008;26(12):3008–3017. [PubMed: 18802032]
18. Topczewska JM, Postovit LM, Margaryan NV, Sam A, Hess AR, Wheaton WW, Nickoloff BJ, Topczewski J, Hendrix MJ. Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. *Nat. Med* 2006;12(8):925–932. [PubMed: 16892036]
19. Strizzi L, Hardy KM, Seftor EA, Costa FF, Kirschmann DA, Seftor REB, Postovit L-M, Hendrix MJC. Development and cancer: at the crossroads of Nodal and Notch signaling. *Cancer Res* 2009;69(18):7131–7134. [PubMed: 19738053]
20. Chen GC, Szakacs G, Annereau JP, Rouzaud F, Liang XJ, Valencia JC, Nagineni CN, Hooks JJ, Hearing VJ, Gottesman MM. Principal expression of two mRNA isoforms (ABCB 5alpha and ABCB

- 5beta) of the ATP-binding cassette transporter gene ABCB 5 in melanoma cells and melanocytes. *Pigment Cell Res* 2005;18(2):102–112. [PubMed: 15760339]
21. Suryo Rahmanto Y, Dunn LL, Richardson DR. The melanoma tumor antigen, melanotransferrin (p97): a 25-year hallmark--from iron metabolism to tumorigenesis. *Carcinogenesis* 2007;28(10):2172–2183. [PubMed: 17449903]
 22. Keshet GI, Goldstein I, Itzhaki O, Cesarkas K, Shenhav L, Yakirevitch A, Treves AJ, Schachter J, Amariglio N, Rechavi G. MDR1 expression identifies human melanoma stem cells. *Biochem. Biophys. Res. Commun* 2008;368(4):930–936. [PubMed: 18279661]
 23. Botelho MG, Wang X, Arndt-Jovin DJ, Becker D, Jovin TM. Induction of Terminal Differentiation in Melanoma Cells on Downregulation of beta-Amyloid Precursor Protein. *J. Invest. Dermatol.* 2009
 24. Hoek KS, Eichhoff OM, Schlegel NC, Dobbeling U, Kobert N, Schaerer L, Hemmi S, Dummer R. *In vivo* switching of human melanoma cells between proliferative and invasive states. *Cancer Res* 2008;68(3):650–656. [PubMed: 18245463]
 25. Hoek KS, Eichhoff OM, Widmer D, Dummer R. Stemming the flood. *Pigment Cell Melanoma Res* 2009;22(1):6–7. [PubMed: 19140883]
 26. Sousa JF, Espreafico EM. Suppression subtractive hybridization profiles of radial growth phase and metastatic melanoma cell lines reveal novel potential targets. *BMC Cancer* 2008;8:19. [PubMed: 18211678]
 27. Elliott AM, Al-Hajj MA. ABCB8 mediates doxorubicin resistance in melanoma cells by protecting the mitochondrial genome. *Mol. Cancer Res* 2009;7(1):79–87. [PubMed: 19147539]
 28. Huang Y, Anderle P, Bussey KJ, Barbacioru C, Shankavaram U, Dai Z, Reinhold WC, Papp A, Weinstein JN, Sadee W. Membrane transporters and channels: role of the transportome in cancer chemosensitivity and chemoresistance. *Cancer Res* 2004;64(12):4294–4301. [PubMed: 15205344]
 29. Folkman J. Angiogenesis: an organizing principle for drug discovery? *Nature Rev. Drug Discov* 2007;6(4):273–286. [PubMed: 17396134]
 30. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996;86(3):353–364. [PubMed: 8756718]
 31. Soeda A, Park M, Lee D, Mintz A, Androutsellis-Theotokis A, McKay RD, Engh J, Iwama T, Kunisada T, Kassam AB, Pollack IF, Park DM. Hypoxia promotes expansion of the CD133-positive glioma stem cells through activation of HIF-1alpha. *Oncogene* 2009;28(45):3949–3959. [PubMed: 19718046]
 32. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, Oh EY, Gaber MW, Finklestein D, Allen M, Frank A, Bayazitov IT, Zakharenko SS, Gajjar A, Davidoff A, Gilbertson RJ. A perivascular niche for brain tumor stem cells. *Cancer Cell* 2007;11(1):69–82. [PubMed: 17222791]
 33. Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, Shi Q, McLendon RE, Bigner DD, Rich JN. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Res* 2006;66(16):7843–7848. [PubMed: 16912155]
 34. Yao X-H, Ping Y-F, Chen J-H, Xu C-P, Chen D-L, Zhang R, Wang J, Bian X-W. Glioblastoma stem cells produce vascular endothelial growth factor by activation of a G-protein coupled formylpeptide receptor FPR. *J. Pathol* 2008;215(4):369–376. [PubMed: 18523971]
 35. Li Z, Bao S, Wu Q, Wang H, Eyler C, Sathornsumetee S, Shi Q, Cao Y, Lathia J, McLendon RE, Hjelmeland AB, Rich JN. Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. *Cancer Cell* 2009;15(6):501–513. [PubMed: 19477429]
 36. Bertolini F, Shaked Y, Mancuso P, Kerbel RS. The multifaceted circulating endothelial cell in cancer: towards marker and target identification. *Nat. Rev. Cancer* 2006;6(11):835–845. [PubMed: 17036040]
 37. Folkins C, Shaked Y, Man S, Tang T, Lee CR, Zhu Z, Hoffman RM, Kerbel RS. Glioma tumor stem-like cells promote tumor angiogenesis and vasculogenesis via vascular endothelial growth factor and stromal-derived factor 1. *Cancer Res* 2009;69(18):7243–7251. [PubMed: 19738068]
 38. Maniotis AJ, Folberg R, Hess A, Seftor EA, Gardner LM, Pe'er J, Trent JM, Meltzer PS, Hendrix MJ. Vascular channel formation by human melanoma cells *in vivo* and *in vitro*: vasculogenic mimicry. *Am. J. Pathol* 1999;155(3):739–752. [PubMed: 10487832]

39. Naumov GN, Bender E, Zurakowski D, Kang SY, Sampson D, Flynn E, Watnick RS, Straume O, Akslen LA, Folkman J, Almog N. A model of human tumor dormancy: an angiogenic switch from the nonangiogenic phenotype. *J. Natl. Cancer Inst* 2006;98(5):316–325. [PubMed: 16507828]
40. Folkins C, Man S, Xu P, Shaked Y, Hicklin DJ, Kerbel RS. Long-term progression and therapeutic response of visceral metastatic disease non-invasively monitored in mouse urine using beta-human chorionadotropin secreting tumor cell lines. *Cancer Res* 2007;67(8):3560–3564. [PubMed: 17440065]
41. Schatton T, Frank MH. Antitumor immunity and cancer stem cells. *Ann. NY Acad. Sci* 2009;1176:154–169. [PubMed: 19796244]
42. Schatton T, Schütte U, Frank NY, Zhan Q, Hoerning A, Robles SC, Zhou J, Hodi FS, Spagnoli GC, Murphy GF, Frank MH. *Cancer Res* 2010;70(2) in press.
43. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. *Nat. Rev. Cancer* 2008;8(4):299–308. [PubMed: 18354418]
44. Tarhini AA, Kirkwood JM, Gooding WE, Cai C, Agarwala SS. Durable complete responses with high-dose bolus interleukin-2 in patients with metastatic melanoma who have experienced progression after biochemotherapy. *J. Clin. Oncol* 2007;25(25):3802–3807. [PubMed: 17761969]
45. Fong L, Small EJ. Anti-cytotoxic T-lymphocyte antigen-4 antibody: the first in an emerging class of immunomodulatory antibodies for cancer treatment. *J. Clin. Oncol* 2008;26(32):5275–5283. [PubMed: 18838703]
46. Kirkwood JM, Tarhini AA, Panelli MC, Moschos SJ, Zarour HM, Butterfield LH, Gogas HJ. Prognostic significance of serum S100B protein in high-risk surgically resected melanoma patients participating in Intergroup Trial ECOG 1694. *J. Clin. Oncol* 2008;26(20):3445–3455. [PubMed: 18612161]

Fig. 1. Cancer stem cell functions. In addition to intrinsic self-renewal, differentiation and proliferative capacity, cancer stem cells can function in angiogenesis/vasculogenic mimicry, host immune modulation, and therapeutic resistance.

Fig. 2.

Cancer stem cell-targeted therapy. Conventional therapies predominantly target tumor bulk population cells, but may spare cancer stem cells. This might result in transient tumor regression, but ultimate tumor relapse (top). In contrast, cancer stem cell-targeted therapies might lead to sustained regression and possibly tumor eradication and patient cures (bottom).