

Cancer Stem- Like Cells in Melanoma Progression, Resistance and Recurrence: Significance for Melanoma Treatment

Review Article

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Abstract

Human malignant melanoma is a highly aggressive tumor which demonstrates heterogeneity and a propensity to drug resistance. Despite improved treatment options, patients with advanced malignant melanoma continue have a poor prognosis as measured by progression-free and overall survival. The cancer stem-like cell (CSC) hypothesis suggests that neoplastic clones are maintained by a small fraction of cells with stem cell properties. As has been demonstrated with other tumor types, melanoma progression, resistance to chemo- and radiotherapy, and recurrence can be attributed to a small fraction of cells termed melanoma stem-like cells (MSCs). These MSCs are characterized by a distinct protein patterns and aberrant signaling pathways, which are either in a causal or consequential relationship to tumor progression, drug resistance and recurrence. This review focuses on the mechanistic role of MSCs leading to tumor progression and metastasis, resistance and recurrence. Understanding the molecular mechanisms underlying MSCs migration, invasion, resistance to standard treatments, and recurrence may help to improve current therapeutic modalities and/or pave the way for the development of new therapeutical management strategy for tumor treatment.

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Introduction

Like most healthy tissues, cancer stem cells are hierarchically organized at a cellular level and exist in the form of small fraction of subpopulations that are primarily responsible for tumor initiation, maintenance and propagation [1-3]. Accordingly, this small fraction of tumor subpopulation is characterized by its ability to drive prolonged maintenance and sustained self-renewal, a mechanism that is essential for tumor growth and differentiation [4,5]. This small fraction of subpopulation drives self-renewal through asymmetrical cell division to produce two daughter cells, one of them is potentially able to differentiate and ultimately forms the tumor mass, whereas the second one maintains its stemness properties so that can function as a CSC [6,7].

The hypothesis of CSC suggests that neoplastic clones are maintained by a small fraction of cells with stem cell properties [8]. Cancers are thought to arise from stem cells and are expected to undergo the same progeny, differentiation and progression observed in normal tissues [9]. Tumor metastatic dissemination, resistance to chemo- and radiotherapy, and recurrence thought to be attributed to CSCs [10-12]. The treatment regimens of melanoma patients are of limited benefit, particularly, in advanced stages since available treatments can primarily target tumor bulk, but not MSCs [13]. The existence of MSCs phenotype has been confirmed in a small fraction of cells derived from either tumor biopsies or from established tumor cell lines [14-17]. These small fractions can form "melanoma spheroids" when allowed to grow in a specific stem cell medium [18] and exhibit self-renewal activity *in vitro* as well as *in vivo* [17-20]. Melanoma spheroids are able to differentiate into different cell types including, those of mesenchymal-lineage besides their tumorigenic potency when transplanted into immune-deficient mice [21,22]. MSCs are characterized by the expression of stem cell maker including, CD20, CD133, and CD166 [17], ABCB5 [23], CD146 [24], and Nestin [25]. More importantly, the increased expression of the self-renewal transcription factor, Bmi-1, in primary and metastatic tumors [26], is an evidence for CSCs potential in tumor progression, invasion and metastatic dissemination. The frequent tumor recurrence seems to result from the preferential killing of tumor bulk while leaving CSCs behind [27]. Although the development of effective therapeutic modalities against peculiar and multiple melanoma antigens still remains a crucial challenge, thus understanding the molecular mechanisms underlying MSCs migration, invasion, resistance to standard treatments and recurrence may help to improve current therapeutic modalities and/or pave the way for the development of new therapeutical management strategy for the tumor treatment.

Malignant Melanoma Characteristics And Epidemiology

Human malignant melanoma is a highly metastatic cancer type that is substantially resistant to standard therapeutic modalities. Although the survival rate of melanoma patients has been improved over the past decades [28], melanoma risk and overall mortality escalate yearly [29]. Generally, primary melanomas are curable by surgical excision when diagnosed early and thereby the number of patients with regional lymph node infiltrations can be decreased [30]. However, the prognosis of patients with visceral metastasis is very poor as evidenced by a median survival rate of only a few months [31]. Because of the therapeutic benefit of current treatment regimens in a significant portion of melanoma patients showing poor prognosis, the development of new therapeutic strategies is urgently needed.

The clinical classification of melanoma is based on the total thickness (in millimeters), the mitotic rate, the presence of ulceration, the depth of penetration, and metastatic location [32]. Whereas, the histological classification defines five distinct stages of melanoma progression including, benign nevi without dysplastic changes, dysplastic nevi, radial-growth phase (RGP), vertical growth phase (VGP), and metastatic melanoma [33].

Although melanoma is derived from melanocyte origin, there are diverse cell types that can be located within or surrounding melanoma lesions. These include endothelial cells, immune cells, keratinocytes, and fibroblasts. Thus, before initiating the malignant transformation, melanocytes start to intersperse among keratinocytes to form an epidermal melanin unit that can transfer melanin granules to the keratinocytes [34]. Whereas, keratinocytes, in turn, serve as the key regulators of early stage melanoma cell homeostasis and proliferation [35].

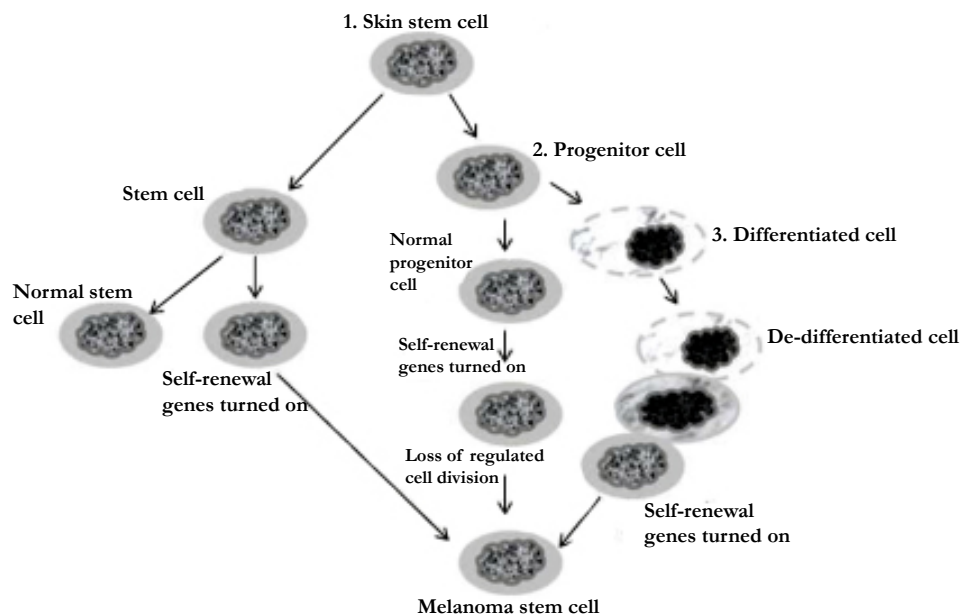
Concept of cancer stem cells (CSC)

The idea that cancer might originate in SC refers back to the 19th

century's concept of "embryonal rests" present in the adult [36-39]. Over a century later, the similarity between the old belief that cancer arises from embryonal rests and the contemporary view that some forms of cancer originate in adult tissue-specific SC has revitalized when leukemia-initiating SC were reported in the peripheral blood of patients with acute myelogenous leukemia (AML) [40]. In the meantime, the presence of SC in breast cancer [41,42], brain cancer [43], ovarian cancer [44], lung cancer [45], colon cancer [46,47], head and neck squamous cell cancer [48] and prostate cancer [49] has been reported. Also, CD20-positive subpopulation has been identified in melanoma [17]. CD20 is an integral membrane protein that is first detected on B-lymphocytes; and is involved in transmembrane calcium flux, and in cell-cycle progression [50]. CD20-positive melanoma cells can grow as non-adherent spheres in human embryonic growth medium, whereas in standard medium they can grow as adherent monolayers. Under appropriate culture conditions, cells from the non-adherent spheres could be differentiated into multiple cell lineages, such as melanocytic, adipocytic, osteocytic, and chondrocytic [51]. These melanoma spheroid cells have been shown to persist after serial cloning *in vitro* and transplantation *in vivo*, confirming their self-renewal ability [17,52].

Accumulating evidence supports the existence and the involvement of CSCs in melanoma initiation, progression, chemoresistance, therapeutic failure and recurrence [17,53,54]. Like normal tissues, melanoma is composed of phenotypically heterogeneous cell populations [55]. Whereas, highly aggressive melanoma subpopulations are characterized by the expression of molecular signatures similar to those of pluripotent stem cells [24]. Thus, the expression of the stem cell markers such as, ABCB5, CD133, CD166, CD34, nestin, c-kit antigens, cancer testis antigens, bone morphogenetic protein (BMP), Notch receptors and Wnt proteins, in addition to their tumorigenic and differentiation potential has been reported in melanoma subpopulation derived from either patients biopsies or from established melanoma cells lines [15,23,56]. A proposed model for the possible mechanism of how cancer stem cells arise is outlined in Figure 1.

Figure 1. Proposed model of how a cancer stem cell does arise. An adult stem cell undergoes genetic and epigenetic alterations, as a consequence progenitor cell can develop to either normal progenitor cell or differentiated cell. The accumulation of a consequent serial of mutations can drive the fully differentiated cell back to gain stemness properties. The activation of self-renewal genes results in the development stem cells to cancer stem cell.



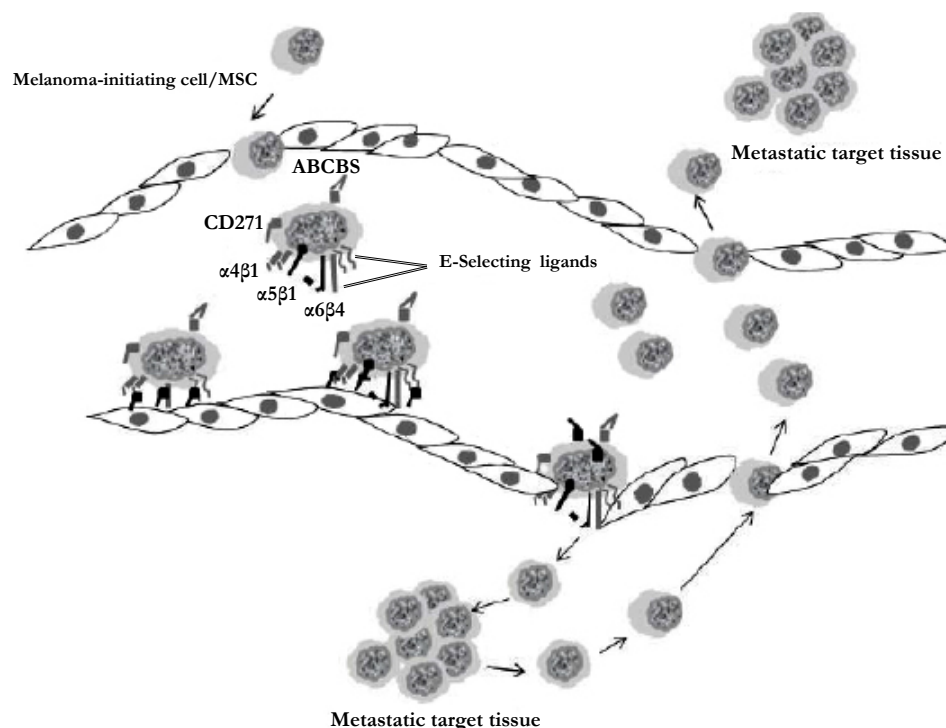
Mechanisms of Cancer Stem-Like Cells in Melanoma Progression and Metastasis

Metastasis is a process, whereby primary tumor disseminates to establish new tumor colonies at distant organ or tissue sites. This process is mediated through the migration into the lymphatic system and/ or dissemination through the blood circulation [57,58]. Accordingly, melanoma is known to be a highly metastatic cancer among solid tumors [59]. Although the clinical significance of melanoma metastasis has been demonstrated, the cellular and molecular mechanisms underlying metastatic melanoma progression are not characterized in detail.

To understand the mechanisms of tumor metastasis, several models and theories have been proposed [60-62]. One of these models based on the clonal selection suggesting that the continuous accumulation of mutations together with the chromosomal imbalances may result in the development of cancerous cells with metastatic properties, particularly, in advanced stages of tumor. Whereas, the other model suggests that the tendency of tumor cells to metastasize thought to be determined in the early processes of tumorigenesis. This theory based on the identification of metastatic features in primary tumor cells or even their transformed precursors before the initiation with the neoplastic progression. Moreover, functional genomic studies revealed that limited subsets of tumor cells within the primary lesions are programmed to metastasize to specific organs [63,64]. Thus, in the context of CSC concept, it is plausible that these limited subsets of tumor cells/subpopulations may be attributed to CSCs, and

thereby have the capacity to initiate tumor formation. Accordingly, some characteristics of the identified CSCs are identical with those of normal stem cells [65]. These stem cell properties are responsible for the mechanisms underlying metastatic process of cancer cells. Also, stromal cell niche or signaling pathways that function as key modulators of normal stem cell proliferation and migration have been shown to play an essential role in tumor cell invasion and dissemination [66,67]. Moreover, the components of cellular niche have been reported to provide anchoring sites for somatic stem cells. As well, the attachment of somatic stem cells to the anchoring sites is orchestrated through mechanisms mediated by distinct signaling molecules such as β -catenin, a downstream effector of Wnt signaling pathway [68,69]. Thus, excessive activation of the Wnt pathway is mainly associated with melanoma progression *via* mechanism mediated by the enhancement of the translocation of β -catenin to the nucleus [70]. The disruption of β -catenin/E-cadherin complexes has been shown to be causally linked to the breakdown of epithelial to mesenchymal transition (EMT) [72]. Thus, the most known key molecules that are associated with melanocyte stem cell maintenance and melanoma metastasis are Slug and Twist [73,74]. These proteins have been reported to play an essential role in the promotion of EMT and the induction of the transcriptional repressors of E-cadherin [75]. Accordingly, the crosstalk between Wnt signaling factors and the cadherin-catenin adhesion system is an essential mechanism that regulates the localization of normal and melanoma stem cells [76]. Also, other molecules such as integrins are involved in the local invasion of melanoma [77] and the regulation of matrix metalloproteinases [78]. Moreover, the melanoma

Figure 2. Proposed model for metastatic dissemination of Melanoma-initiating cells/melanoma stem-like cells (MSC). The circulation of MSC allows it to roll in the blood flow on microvascular endothelial cells of the metastatic target tissue. As a result the constitutively active MSC $\alpha 4\beta 1$ integrin binds to the endothelial VCAM-1, and the E-selectin glycoprotein and glycolipid ligands of MCS bind to the endothelia E-selectin of microvascular endothelia cells. Also, the elevated $\alpha 5\beta 1$ integrin on MSC can interact with the endothelial ligand, fibronectin (FN). As consequence, the MSC becomes able to traverse endothelial cell-cell junction through $\alpha 5\beta 1$ and the $\alpha 6\beta 4$ binding to the surface and basement membrane ligands, Laminin (LN) and FN. The process of MCS migration is thought to involve the binding of IL-8 and SDF1 to CXCR1 and CXCR4, respectively. A mechanism that is described in migratory process of melanoma and CSCs. Also, additional factors such as VEGFR-1, VE-cadherin, and TIE may be involved in the promotion of MSC metastasis.



metastasis gene, NEDD9 is required for melanoma cell invasion and dissemination. The expression of NEDD9 protein is elevated in patient biopsies and is associated with melanoma progression [79]. The detection of NEDD9 protein in CD133⁺ human cord blood progenitor cells under condition leading to the enhancement of metastatic potential of cancer cells, suggesting an essential role for this protein in the regulation of the metastatic potential of CSCs. Thus, during tumor progression CSCs hijack the mechanistic program of the epithelial-to-mesenchymal transition, releasing their epithelial characteristics, such as resistant cadherin-dependent junctions to gain the invasive ability [80]. The ability of normal and tumor cells to migrate is based on a common regulatory mechanisms, by which the stromal cell derived factor 1 (SDF-1) signaling mediates the regulation of hematopoietic stem cells (HSCs) migration, *via* the C-X-C chemokine receptors type4 (CXCR4). Like other cancer types, the dissemination of melanoma subpopulation is mediated by SDF-1/CXCR4 system [81]. Thus, the inhibition of the interaction between CXCR4 and its ligand SDF-1 has been reported to suppress melanoma metastasis into murine lungs [81]. Of note, the elevation of CXCR4 levels is mostly associated with poor prognosis in patients with malignant melanoma [82]. Accordingly, the existence of CD133⁺CXCR4⁺ melanoma subpopulation in cutaneous melanomas with invasive phenotype and poor prognosis is an evidence for the functional role of SDF-1/CXCR4 system in the promotion of melanoma progression and migration, and finally the metastatic potential of MSCs. Proposed model for metastatic dissemination of Melanoma-initiating cells/ MSC is out lined in Figure.2.

Mechanisms of Melanoma Stem-Like Cells- Mediated Chemo- Resistance

The chemo-resistance of advanced malignant melanoma is mediated by several mechanisms, which have been intensively studied in the context of tumor stem cell [83]. These resistance mechanisms include the destruction of cancer apoptotic pathways, excessive activation of aberrant signaling pathways leading to tumor growth and survival, induction of the expression of ABC drug efflux transporters [84,85]. ABCB5, is a human multi-drug resistance (MDR) P-glycoprotein family member, which mediates chemo-resistance in melanoma *via* its function as a drug efflux transporter [86,87]. The expression of ABCB5 is associated with increased level of tumor antigen P97, a melanotransferrin (MTf) that is associated with melanoma growth [88]. More importantly, the expression of ABCB5 has been shown to be specific to CD133⁺ tumor stem cell phenotype [14]. Also, immune histochemical analysis of clinical samples derived from patients with malignant melanomas showed that the expression of ABCB5 is characteristic for a minority of melanoma population of both primary and metastatic origin [89].

Because of CSCs are resistant to chemo- and radiation therapy, the failure of conventional therapy and thereby result in tumor relapse. Accordingly, tumor recurrence may be the consequence of the preferential killing of differentiated cells while leaving CSCs behind. Thus, understanding the mechanisms that underlying MSCs resistance may help improve the treatment outcome and, in turn, prevent melanoma recurrence.

Chemo-and radiation resistance of CSCs is mediated through genetic and cellular alterations conferring resistance to conventional therapeutic approaches [90,91]. Genetic and cellular alterations include, tumor dormancy associated with delayed cell cycle kinet-

ics, efficient DNA repair, the expression of multidrug-resistance transporters, and destruction of the apoptotic pathway[92]. The role of checkpoint kinases 1 /2 (Chk1/2) in the modulation of CSCs resistance to chemo- and radiation therapy has been reported [93,94]. It has been reported that Chk1/2 kinases have higher basal and inducible activities in CSCs when compared to normal stem cells [95]. Although some therapeutics like alkylating agents have been approved for their cytotoxicity, these agents can also enhance Chk1/2 kinases in CSCs and thereby contribute to the potentiation of the resistance mechanisms of CSCs to chemo- and radiation therapy [96]. Moreover, CSCs can deduce resistance to chemical agents through the expression of drug efflux pumps such as ABC family members, as a mechanism that responsible to pump the drugs out of the cells [97]. The expression of ABCB5 in CSCs including MSCs has been reported [98]. The role of Akt pathway in the modulation of CSCs resistance is also suggested [99]. Also, the expression of apoptotic inhibitors is a mechanism, whereby CSCs confer resistance to chemo-and radiation therapy.

Mechanisms of Cancer Stem-Like Cells in Melanoma Recurrence

The ability of normal adult stem cells to maintain the balance between self-renewal and differentiation is mediated by adhesive factors and signaling interactions in stem cell niches. Thus, the mechanism regulating the entry of metastasis-initiating cells into dormancy and to undergo reactivation of key components of cancer stem niche [100,101].

Melanoma recurrence is an important phenomenon, since the presence of persistent tumors in patients is important not only for clinical management, but also for understanding tumor biology, particularly tumor dormancy. Melanoma recurrence and dormancy seem to result from the inability of current therapeutic regimens to completely eradicate the putative melanoma subpopulation [102]. Although the recurrence risk is very low so that the need to invasive follow up beyond long time is not taken seriously, and new symptoms or clinical findings may go back to the earlier diagnosis.

Apart from clinical cause and features of melanoma recurrence, the balance between cell proliferation and apoptosis is thought to be an essential determinant of the melanoma tumorigenic potential. Although the proliferative activity of melanoma is regulated, in part, by tumor vascularity[103], the contribution of nonvascular extracellular matrix is also essential for tumor progression[104,105]. Accordingly, the role of MSCs is increasingly recognized in the progression of primary melanoma and its metastasis [24,54]. Accumulated evidence reveals that the cells that initiate metastatic outgrowth of CSCs are tumor cells with stem cell properties [54]. The antigenic patterns of primary melanoma are variable and heterogeneous, and possess small fraction of subpopulation that is characterized by the expression of CSC marker, such as CD166, CD133, and nestin [24], an evidence for the presence of genetic pathways that are instrumental for stem cell biology. As widely established metastasis-initiating cells are cancer stem cells, whose entry into dormancy and subsequent reactivation is mediated by intrinsic programs and signaling pathways that resemble the self-renewal mechanism of adult stem cells. Thus, many patients with carcinomas have been found to suffer from metastatic recurrence in later years after initial diagnosis and radically surgical and nonsurgical treatments [106,107].

Mechanisms Regulating The Adhesion of Melanoma Stem-Like Cells To Their Niches

Normally, at least in the hematopoietic, intestinal, and hair follicle systems, the niche maintains SC primarily in a quiescent state by providing signals that inhibit cell growth as evidenced by the ability of SC to retain bromodeoxyuridine labeling for long periods of time [108] The function of niche cells is likely to block expression of genes that trigger the onset of differentiation with differentiation being the default stage (fail-safe mechanism). The proliferation of CSC needs depends on the reactivation of stem cell (SC) niche in response to CSC self-renewal signals. Alternatively, CSC or transient-amplifying progenitor cells have become niche-independent upon genetic or epigenetic alterations that enable autonomous self-renewal.

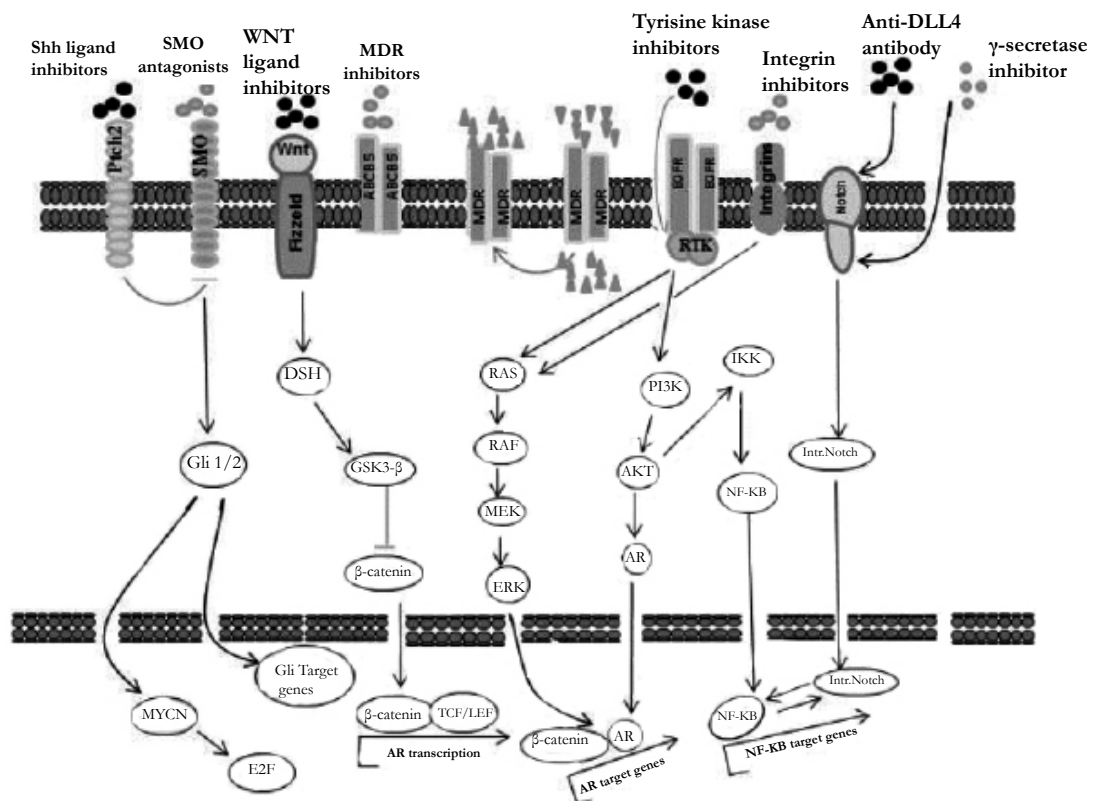
Cadherin and β -catenin adhesion molecules anchor melanocytes in the epidermis [109]. Epidermal SC remain attached to the niche through the cadherin- β -catenin interaction, while in the activated stage, β -catenin is localized to the nucleus, a mechanism that is essential for SC proliferation to ensue [110]. Besides the cadherins/catenins, multiple other signaling and adhesion molecules are involved in niche regulation such as SCF/c-Kit, Jagged/Notch, angiopoietin-1/Tie2 (Ang-1/Tie2), and Ca^{2+} -sensing receptor (CaR) [111,112].

Strategies to Eradicate CSC

As highly differentiated cells that rarely divide, and as rapidly proliferating cells that have poorly differentiated phenotypes, two basic therapeutic approaches for combatting cancer have developed: "differentiation therapy" [113] to induce differentiation and "destruction therapy" [114] to thwart malignant proliferation. Although the suggested therapeutic strategies are theoretically promising, the limited success in some cases encouraged the researchers and clinicians to propose a new strategy to eradicate CSC.

The concept of CSC helps to explain why treatments that substantially reduce the tumor mass by removing proliferating cells fail to cure patients, because CSC are usually slow cycling and, therefore, resistant to the conventional treatments in addition to displaying enhanced DNA repair [115,116] and asynchronous DNA synthesis [117]. In addition, SC expresses higher levels of anti-apoptotic proteins such as members of the Bcl-2 family. Further, most CSC express high levels of multiple drug resistance proteins (MDR) and, thus, are able to pump out many chemotherapeutic drugs. Lastly, effective immunologic reactions to cancer antigens may only target differentiating tumor progeny whereas CSC most often do not express tumor markers and are therefore not attacked by the immune system. If CSC comprises only a minor fraction of total tumor cells and if these cells drive tumorigenesis, then profiling of purified populations of CSC may

Figure 3. Schematic overview of signaling pathways that can function as therapeutic target MSC. Therapeutic strategies for the efficient treatment of melanoma metastasis. The inhibition of Sonic Hedgehog (Shh) ligand to bind to its receptor Patched 1 (Ptch1) by the Shh ligand inhibitors leads to the repression of the smoothed (Smo) that becomes unable to promote the activation of Gli proteins (Gli1/2) that can not be translocated the nucleus, where they can function as transcriptional activators of their target genes that are essential for self-renewal of tumor-initiating cells/MSCs. Also, direct inhibition of Smo by Smo antagonists can inhibit self-renewal of MSC. The neutralization of Wnt ligand by Wnt ligand inhibitors leads to the suppression of Wnt pathway that, in turn, inhibits tumor progression. The inhibition of tyrosine kinase receptors such as EGF by tyrosine kinase inhibitors, integrins by integrin inhibitors, or Targeting of Notch pathway by anti-DLL4 antibody or with γ -secretase inhibitors can be an efficient strategies to eradicate MSCs.



identify more successful molecular targets than profiling the bulge of the tumor. In this regard, a cancer signature of 11-genes has been shown to be under the control of the SC self-renewal gene Bmi-1 [118]. This conserved Bmi-1-driven pathway correlated with earlier recurrence, distant metastases and death in 1153 cancer patients based on the analysis of 11 genes [118]. From this and other studies, diverse hormones, growth factors, cytokines and chemokines (androgens, estrogens, EGF and TGF- β -catenin, Notch, BMP4, TGF- β /CXCR4) and tumorigenic signaling elements (telomerase, PI3K/AKT, NF- κ B and Myc-1) have been suggested as important therapeutic targets to tackle initiation and progression of various cancers [119-121]. Therefore, it is becoming increasingly clear that effective cancer therapeutics may have to be retargeted to CSC.

Although the significant regression of bulky tumor lesions in response of patients to the treatment with inhibitors specific to BRAF, in addition to tumor recurrence many of melanoma patients develop resistance to treatments [122,123]. Therefore, the development of therapeutic strategies based on the combination of multi-modal therapeutic modalities that specifically trigger the destruction multiple pathways that are responsible for the maintenance of the bulk of the tumor with inhibitors that can trigger multiple pathways that are specific for melanoma subpopulation may be essential to prolonged disease free survival melanoma patients. However, the profile of melanoma patients before and after therapy may help determine and optimize the best combination therapeutic strategy for the treatment of individual melanoma patients. Thus, the analysis of drug resistant melanoma subpopulations that are selected after therapy for epigenetic and or phenotypic alterations may help design personalized therapeutic approach for the treatment of individual patients. Figure 3 outlines signaling pathways and their possible targets as therapeutic strategies to eradicate MSC.

Conclusion

Malignant melanoma is a highly aggressive and heterogeneous tumor that composed of many subpopulations with unique genotypic and phenotypic pattern. The progression, maintenance, resistance to chemo- and radiotherapy, and recurrence of melanoma is attributed to small fraction of melanoma subpopulation that is similar to adult stem cells in characteristic and behavior. The identification of melanoma stem cells (MSCs) improved our knowledge regarding melanoma initiation, progression resistance, and recurrence. The improvement of current therapeutic strategies and/or their substitution by more efficient one, may help to improve the treatment outcome of melanoma. The investigation of the molecular mechanisms, which are responsible for the regulation of tumor progression, resistance and recurrence are attributed to MSCs. Understanding these mechanisms will help identify unique and/or multiple pathways that may be a potential target for melanoma treatment. The destruction or excessive activation of these pathways by small molecules inhibitors will help not only to eradicate the tumor bulk, but also will help eliminate melanoma-initiating cells. The ability to design therapeutic strategies based on elimination of both tumor bulk and MSCs is urgently needed for an efficient treatment of malignant melanoma.

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