Effect of melanoma stem cells on melanoma metastasis (Review)

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Abstract. Cancer stem cells (CSCs) are involved in the metastatic process, the resistance of many types of cancer to therapeutic treatments and consequently the onset of recurrences. The CSC concept therefore significantly extends our understanding of melanoma biology. More recently, melanoma stem cells (MSCs) have been described in melanoma as expressing specific biomarkers. These primitive melanoma cells are not only capable of self-renewal and differentiation plasticity, but may also confer virulence via immune evasion and multidrug resistance, and potentially, via vasculogenic mimicry and transition to migratory and metastasizing derivatives. This review will present the specific biomarkers of MSCs, including CD133, ATP binding cassette subfamily B member 5, CD271, CD20 and aldehyde dehydrogenase, which can regulate the transduction of tumor-related signals. These signal molecules can reversely act on tumor cells and regulate tumor angiogenesis, leading to the occurrence of melanoma metastasis. Targeting these specific biomarkers could inhibit the progression of melanoma and may help the development of novel therapeutic strategies for melanoma.

Contents

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1. Introduction

Melanoma is a malignant tumor of melanocytes that typically arises in the skin. It is highly malignant, prone to metastasis and recurrence, accounting for ~75% of skin cancer-associated mortality (1). The treatment of advanced or metastatic melanoma is particularly challenging, and there is a high tendency for patients to relapse and become resistant to current therapeutic agents (2). Although molecular targeted therapy and immunotherapy have been reported to prolong the survival time of patients, most patients will develop drug resistance within one year (3-5), resulting in melanoma metastasis. The existence of melanoma stem cells (MSCs) is one of the potential causes of melanoma invasion and metastasis.

Cancer stem cells (CSCs) have been shown to be an integral part of solid tumors (6). Furthermore, CSCs exhibit distinctive and remarkable capacities of self-renewal, differentiation and proliferation, which are believed to have a key role in all aspects of carcinogenesis, including tumor recurrence and metastasis (7,8). Previous studies have demonstrated that the essence of tumor metastasis is the transfer and homing of CSCs (6,9). In the last decade, with the rise of CSCs, several lines of evidence suggested that CSCs may be at the origin of tumor metastasis (10-12). Interestingly, the CSC subpopulation is responsible for many aspects of tumorigenesis and has been reported to serve a crucial role in melanoma development, progression, drug resistance and metastasis (13,14). The fact that the CSCs are resistant to chemotherapy also explains that traditional anticancer drugs can only inhibit or narrow the tumor, but not completely eradicate it, leading to tumor metastasis and recurrence (15,16). In addition, CSCs have been reported to express a variety of biomarkers, such as CD34, aldehyde dehydrogenase 1 (ALDH1), CD271, CD44 and lysine demethylase 5B (JARID1B); however, none of these markers have been shown to be CSCs-specific (17-19). Several potential biomarkers of CSCs have been demonstrated to be expressed by certain human solid tumors such as melanoma (16), including CD133, ATP binding cassette subfamily B member 5 (ABCB5), CD271, CD20 and ALDH (Table I). Although the mechanism of MSCs promoting tumor metastasis and recurrence has not been fully elucidated (13,20,21), the activation of the signaling pathways, including Notch, Hedgehog and Wnt, is modulated by

these biomarkers to maintain the characteristic of MSCs, thus promoting angiogenesis and epithelial-mesenchymal transition (EMT) of melanoma and accelerating tumor metastasis (7,20,22) (Fig. 1). It is therefore important to determine the role of MSCs in the invasion and metastasis of melanoma.

2. Melanoma metastasis is modulated by molecular markers of MSCs

CD133. As a surface protein with unknown function, CD133 (prominin-1) can be expressed on human melanoma, but hardly be detected in normal skin (23). It has been reported that CD133, a stem cell-related surface antigen, is closely related to tumor proliferation and progression in various types of tumor, including melanoma (15,24,25). Furthermore, $CD133^+$ melanoma cells exhibit 411 upregulated genes, which are associated with angiogenesis, adhesion and migration (26). Melanoma $CD133^+$ CSCs have the potential to initiate tumor progression. In addition, CD133 can activate MAPK signal pathway through notch receptor 1 (Notch1), regulate the expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinases and promote the interaction of tumor endothelial cells, leading to the increase of tumor angiogenesis and lung metastasis (26). Furthermore, the expression of CD133, p-p38 and p-MEK3/6 in metastatic melanoma is significantly higher than that in paracancerous tissues (26,27), suggesting that Notch1 and its MAPK signaling pathway network might be considered as potential targets for MSCs-mediated melanoma targeted therapy (28,29). Furthermore, it is generally known that tumor angiogenesis is an essential factor for tumor growth and metastasis. In particular, $CD133^+$ and $ABCB5^+$ MSCs are involved in the formation of perivascular niches (30). When RNAi is used to block the expression of CD133, the tumorigenicity of stem cells *in vivo* is significantly decreased and the expression of CD144 and ABCB5 that are closely related to the vascular microenvironment is downregulated (30). However, $ABCB5^-$ cells lose the ability to form $CD144^+$ angiogenic mimicry. It has been reported that $CD133^+/ABCB5^+$ MSCs exist in $CD144^+$ angiogenic mimicry, suggesting that $CD133^+$ MSCs could promote tumor metastasis by increasing the formation of angiogenic mimicry and specific vascular microenvironment (30). Zimmerer *et al* (31) demonstrated by fluorescence microscopy that $CD133^+$ melanoma D10 cells xenograft into nude mice can trigger an important angiogenesis process. A clonal dominance of a $CD133^+$ population exists within the hierarchy of cells in cutaneous tissues from patients that have undergone successive progressive stages of melanoma, from primary to metastatic lesions (32). In addition, in the $CD133^-$ melanoma cells subpopulation, exposure to taxol induces the activation of apoptosis signal-regulating kinase1/c-jun-N-terminal kinase, p38 and ERK pathways and Bax expression; however, in $CD133^+$ cells, taxol only enhances the activity of the ERK pathway (33). Furthermore, it was demonstrated that the expression of $CD133^+$ in patients with recurrent and metastatic melanoma is twice higher than in patients with primary melanoma (32). Mechanistically, CD133 downregulation in human metastatic melanoma cells can decrease the capacity of sphere-forming and the metastasis potential of melanocytes (28). In addition, CD133, is closely related to the expression of certain tumor associated antigens and could thus serve as a potential target for immunotherapy (34,35). CD133 may therefore be considered as a predictive marker of melanoma and as a potential therapeutic target of high-risk melanoma.

ABCB5. ABCB5 is a member of the ATP binding cassettes (ABC) transporter family and a regulator of cell membrane potential. ABCB5 can regulate the fusion of normal skin progenitor cells and is considered as one marker of MSCs (36,37). It is now well accepted that ABC transporters mediate multidrug resistance through drug efflux in cancer cells, which is usually related to cancer stem cells (38-40). *In vivo* genetic lineage tracking demonstrated a specific capacity of $ABCB5^+$ sub-populations for self-renewal and differentiation, as $ABCB5^+$ cancer cells generate both $ABCB5^+$ and $ABCB5^-$ progeny whereas $ABCB5^-$ tumor populations give rise, at lower rates, exclusively to $ABCB5^-$ cells (37). Subsequently, ABCB5, a marker of MSCs, has high tumorigenicity potential and is co-expressed with other MSCs markers, such as CD133 (39,41,42). $ABCB5^+$ tumor cells detected in human melanoma patients show a primitive molecular phenotype and correlate with clinical melanoma progression (43). Analysis of some clinical data demonstrated that overexpression of ABCB5 can promote tumor progression, and that ABCB5 expression is usually low in pigmented nevus subpopulation but high in primary and metastatic melanoma cell subpopulation (37,44). In serial human-to-mouse xenotransplantation experiments, $ABCB5^+$ melanoma cells possess greater tumorigenic capacity than $ABCB5^-$ bulk populations (44,45). Ma and Frank (45) reported that $ABCB5^+$ melanoma cells exist in the peripheral blood of patients with melanoma. Subsequently, transplanting these cells to Nod/SCID/IL2 mice can induce distant metastasis, and the degree of metastasis is directly proportional to the number of $ABCB5^+$ melanoma cells transplanted.

Frank *et al* (46) demonstrated that $ABCB5^+$ human melanoma cells promote the formation of angiogenic mimicry by expressing endothelium-specific markers and other angiogenic proteins. Because angiogenic mimicry plays an essential role in angiogenesis of melanoma (21), the $ABCB5^+$ subpopulation of human melanoma expresses preferentially the proteins tyrosine kinase with immunoglobulin like and EGF like domains 1 and CD144 (VE-cadherin) and other angiogenic differentiation markers. Furthermore, $ABCB5^+$ and $CD133^+$ MSCs express preferentially VEGFR1 and VEGF, which are important for the angiogenic mimicry of human melanoma cells and further promote melanoma metastasis (26,46). It was reported that *in vivo* targeting of VEGFR-1 blocks the development of $ABCB5^+$ vascular mimicry and inhibits tumor growth in melanoma (46). Furthermore, one potential mechanism of metastasis promotion and melanoma recurrence by ABCB5 is that ABCB5 might inhibit the activation of T cells and contribute to immune escape of tumor cells (47). Furthermore, the levels of MHC class I receptor, melanoma associated antigen, costimulatory molecule B7.2 and PD-1 are lower in $ABCB5^+$ cells than in $ABCB5^-$ cells (48). These findings indicate that targeting ABCB5 MSCs markers in the treatment of melanoma might be beneficial, ongoing clinical trials have proved this view (48).

CD271. CD271, also known as low affinity nerve growth factor receptor or p75NTR, is a characteristic marker of MSCs (36,49). High Expression of CD271 has been reported

Table I. The role of specific biomarkers in melanoma.

CD133	CD133$^+$ melanoma stem-like cells confer resistance to taxol (33).
	CD133 and ABCG2 positive melanoma cells have the potential ability to promote tumorigenesis (27)
	CD133$^+$ population within cutaneous tissues promotes the continuous progress of melanoma (32)
	CD133 is a melanoma immunogenic target of which expression is often associated with expression of cancer/testis antigens (34,35)
ABCB5	Expression of ABCB5 in melanoma cells promotes resistance to chemical agent (40)
	ABCB5$^+$ tumor cells detected in human melanoma patients show a primitive molecular phenotype and correlate with clinical melanoma progression (37)
	ABCB5 interacts with CD166, which promotes clinical malignant melanoma progression (39)
CD271	CD271 is associated with neurotrophins and their receptors, which plays an important role in promoting melanoma cell invasion *in vitro* and migration (52,55)
	A relatively high frequency of CD271/Sox10-positive cells correlates with higher metastatic potential and worse prognosis in human melanoma (65)
	CD271$^+$ cells show higher tumorigenicity and metastatic ability in melanoma (49)
	The low affinity neurotrophin receptor CD271 plays a dual role as a mediator of phenotype switching, suppressing melanoma cell proliferation while concomitantly promoting metastasis formation *in vivo* (58).
CD20	Melanomas contain distinct cell subpopulations including one expressing CD20 with stem cell-like and tumor-initiating characteristics (36,72).
	The CD20-expressing melanoma subpopulation is characterized by self-renewal, differentiation into several cell lineages and high tumorigenicity (73).
ALDH	ALDH1 is a promising new marker for cancer stem cells and catalyzes the oxidation of intracellular aldehydes, thus conferring multidrug resistance (83).
	ALDH1 is important for cell proliferation, survival and resistance to chemotherapeutic agents (85,86).
	ALDH$^+$ melanoma cells are more tumorigenic than ALDH- cells in both NOD/SCID mice and NOD/SCID/IL2rγ^{null} mice (88).
Sox10	Loss of Sox10 impairs neural crest stem cell maintenance and reduces the number of CD271-positive cells and counteracts tumorigenesis in melanoma (92,93).
	Following Sox10 knockdown, human melanoma cells are no longer able to initiate tumors in a xenotransplantation model (67).

ABCB5, ATP binding cassette subfamily B member 5; ALDH, aldehyde dehydrogenase; Sox10, SRY-Box transcription factor 10.

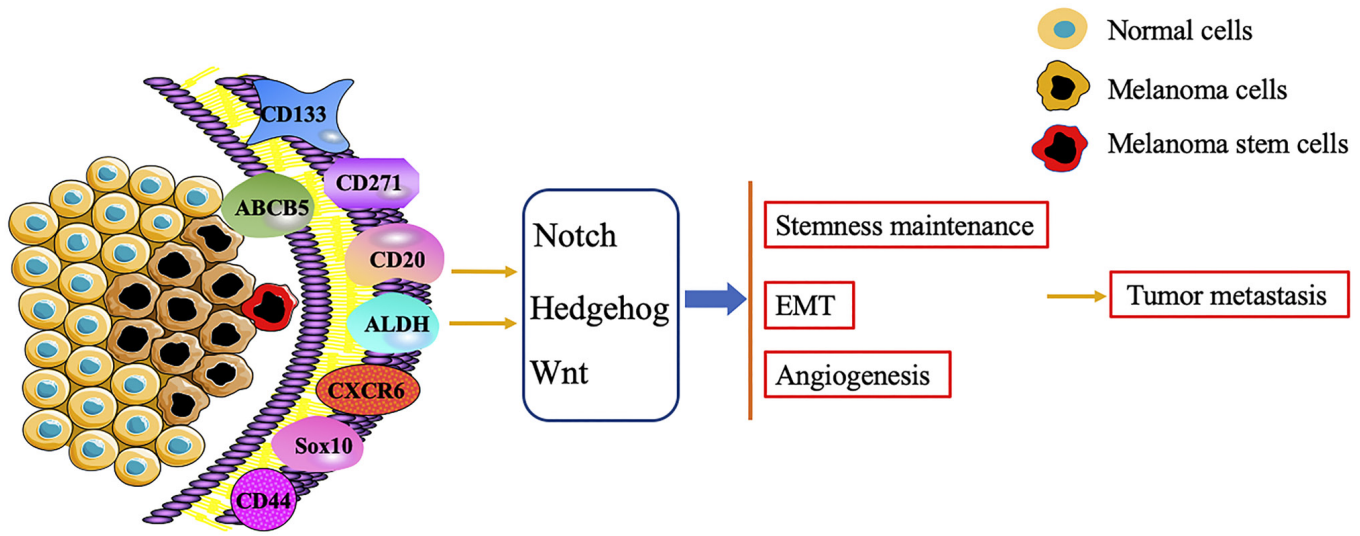

Figure 1. Melanoma stem cells account for only a small part of melanoma cells, which express a variety of cell markers, such as CD133, ABCB5, CD271, CD20, CXCR6, Sox10, CD44 and ALDH. Activation of the signaling pathways (including Notch, Hedgehog and Wnt) is modulated by these markers, in order to maintain the characteristic of melanoma stem cells, thus promoting EMT and angiogenesis of melanoma and accelerating tumor metastasis. ABCB5, ATP binding cassette subfamily B member 5; ALDH, aldehyde dehydrogenase; CXCR6, C-X-C motif chemokine receptor 6; EMT, epithelial-mesenchymal transition; Sox10, SRY-Box transcription factor 10.

in numerous human neural-crest-derived tissues and in some human cancers, including melanomas (50-52). Previous studies demonstrated that $CD271^+$ melanoma cells have a higher tumorigenicity potential than $CD271^-$ cells and are involved in the metastasis of melanoma *in vivo*, especially in peripheral nerves (49,53-55). CD271 is the most reliable cell surface marker for the identification of melanoma heterogeneous subsets (49). In addition, not only CD271 marks dedifferentiated melanoma cells emerging, for instance, through TGFβ-mediated EMT, BRAF inhibitor-induced reprogramming (56) or in response to immunotherapies (57), but it is also functionally involved in promoting low rates of proliferation and high metastatic capacity (58). In fully immunocompromised mouse models, including NOD/SCID/IL2rγ^{null} mice, melanoma cells expressing the neurotrophin receptor CD271 have a higher tumor-initiation capacity than $CD271^-$ cells, although the negative fraction is also able to generate tumors in this mouse model (49). Furthermore, $CD271^+$ melanoma cells are prone to form liver and lung metastasis in mice, whereas $CD271^-$ melanoma cells rarely form metastasis (59). In human samples, the proportion of CD271/ SRY-Box transcription factor 10 (Sox10) positive cells is significantly increased in metastatic melanoma compared with primary melanoma cells (49,60). Interestingly, CD271 inactivation not only results in decreased melanoma cell survival, but also in increased sensitivity to BRAF inhibitor treatment, suggesting that CD271 might confer therapy resistance (61). Schnegg *et al* (62) demonstrated that $CD133^+$ and $CD271^+$ MSCs accumulate in the perivascular niche that melanoma cells with formation of angiogenic mimicry positively express CD271. Similarly, in the human uveal melanoma cell line c918 cultured in 2D and 3D cultures, evaluation of CD271 expression through immunofluorescence showed that CD271 is expressed on the tumor cells that form the vasculogenic mimicry (63). It was confirmed that melanoma cells that form the vasculogenic mimicry acquire the tumor stem cell-like phenotype and participate in angiogenesis (63).

A previous study reported that the number of MSCs positive for ABCB5, CD271 and receptor activator of nuclear factor κ B in circulating tumor cells (CTCs) of patients with advanced melanoma is significantly increased (64). In addition, CTCs are highly enriched in MSCs, which is a vital parameter inducing the formation of distant secondary tumors (64). Furthermore, melanoma-associated antigens, such as melanoma antigen recognized by T-cells 1, are less exposed to CD271 and ABCB5 positive melanoma cells, which supports the hypothesis that melanoma cells expressing the MSCs makers can escape the attack of host immune system (65). A previous study demonstrated that CD271 overexpression in melanoma cells inhibits the production of melanoma-specific cytotoxic T lymphocytes (CTLs), and that interferon-γ from CTLs subsequently triggers the expression of CD271 in melanoma cells, downregulating therefore the production of melanoma antigens (66). Similarly, it was reported that melanoma cells highly expressing CD271 are associated with high tumor metastasis potential and poor prognosis of patients (67).

CD20. In melanoma, numerous subpopulations with the capacity of self-renewal, differentiation, tumorigenicity and/or drug resistance have been described (49,68-70), including one subpopulation expressing the B cell marker CD20 (36,71-73). Further characterization with respect to melanoma-associated antigen indicated that a more primitive melanoma phenotype revealed overexpression of CD20 (16). Importantly, CD20 was initially identified on a small percentage of human melanoma cells when cultured in embryonic stem cell medium and found on nonadherent spheres. These $CD20^+$ melanoma cells with the ability of self-renewal and differentiation followed the definition of tumor stem cells. Consistent with the view that cancer stem cells occupy a small part of tumors, $CD20^+$ cells only account for ~2% of the total number of melanoma cells (74). However, $CD20^+$ melanoma cells were demonstrated to be highly tumorigenic *in vivo* following xenotransplantation, suggesting that these cells exhibit tumor-initiating capacity (16). A previous study reported that the melanoma cells WM115 in the non-adherent form (melanoma spheroid cells) express a higher level of CD20 compared with adherent WM115 cells and that it is more likely to develop tumor when melanoma spheroid cells are transplanted into mice (16). Similarly to all stem cells, melanoma spheroid cells are also capable of proliferation, differentiation and self-renewal (16). In the late stage of metastatic melanoma, the effect of targeted therapy with anti-CD20 monoclonal antibody is more significant than that of non-targeted therapy and can even achieve a clinical complete response (75-77). In melanoma patients resistant to chemotherapeutic drugs, intratumoral injections of rituximab, which is the specific antibody against CD20, induces a regression of tumor growth, accompanied by a significant decrease in serum levels of inflammatory markers (77). Although there is no clear evidence that $CD20^+$ MSCs are directly involved in melanoma metastasis, the stem cell-like characteristics of $CD20^+$ melanoma cells have been confirmed, and their high tumorigenicity and migration ability are the main reasons for tumor progression (16,76). CD20 may therefore be considered as a new target for melanoma treatment in the future.

ALDH. ALDH represents a group of isoenzymes that can oxidize acetaldehyde to acetic acid. The enzymatic activity of ALDH has been used to identify stem or progenitor cells from various malignancies including breast, colon and lung cancers (78-80). According to previous studies, ALDH, which is a marker in many CSCs (78), is associated with multidrug resistance and immune tolerance of different types of solid tumor (78,81-83), can inhibit oxidative stress and enhance resistance to chemotherapeutic drugs, such as oxazolidine, taxanes and platinum drugs (84-86). It has been reported that melanoma cells with high expression of ALDH exhibit MSCs characteristics (87,88). Furthermore, ALDH-positive melanoma cells are more resistant to chemotherapeutic agents, and silencing ALDH1A using small interfering RNA can sensitize melanoma cells to drug-induced cell death (88). $ALDH^{high}$ cells (melanoma cells with high expression of ALDH) can produce more melanoma clone spheres than $ALDH^{low}$ cells (melanoma cells with low expression of ALDH) (87); however, the ability of melanoma formation *in vivo* is significantly inhibited following ALDH silencing (88). For example, following downregulation of aryl hydrocarbon receptor (AHR) and/or ALDH1 in mouse melanoma B16F10 cells through retroviral transduction, Contador *et al* (89) demonstrated that ALDH1 downregulation could inhibit the metastasis of melanoma cells without AHR expression,

reduce the number of $CD133^+/CD29^+/CD44^+$ cells and the size of melanospheres, confirming that overactivation of ALDH1 could promote the progression of melanoma in the context of AHR deficiency. Similarly, ALDH1 silencing in melanoma cells using short hairpin (sh)RNA significantly delays the appearance and growth of xenograft melanoma and dramatically decreases the number and load of metastases in mice (90). These studies confirm that targeting ALDH1 may be considered as an effective strategy for the treatment of advanced melanoma.

Other molecular markers of MSCs. Sox10 is a key nuclear transcription factor involved in the malignant transformation of melanocytes that has the potential to be a marker of MSCs (91). The positive rate of Sox10 in sentinel lymph node micrometastasis of melanoma is close to 100%, which is significantly higher than other melanoma markers responsible for melanoma metastasis, such as S100, HMB45 and Melania (91). Furthermore, it has also been reported that the stem cell function of $CD271^+$ melanoma cells may be correlated to the CD271/Sox10 interaction network, although the underlying mechanism remains unclear (59,92). High levels of organic cation transporter (OCT)3/4, Nanog homeobox (Nanog) and Sox10 are found in $CD133^+$ transgenic mice and human melanoma cells, and were demonstrated to promote tumor neovascularization, indicating that Sox10 and other MSCs markers can regulate each other and accelerate tumor progression (26). However, Sox10 silencing in human melanoma cells suppresses neural crest stem cell properties, inhibits cell proliferation and survival and completely abolishes *in vivo* tumor formation (93). Sox10 may therefore represent a promising target for the treatment of congenital naevi and melanoma in human patients.

C-X-C motif chemokine receptor 6 (CXCR6) is also an important marker of MSCs. Compared with ATP binding cassette subfamily G member 2 $(ABCG2)^+$ melanoma cells, $CXCR6^+$ melanoma cells can produce larger tumors in a shorter time, and $ABCG2^+$ and $CXCR6^+$ double-positive cells have a higher tumorigenic potential than $ABCG2^+$ or $CXCR6^+$ single-positive melanoma cells in promoting tumor metastasis (94).

CD44 has been used as a specific marker of MSCs in preclinical previous studies (95,96). It has been reported that blocking insulin-like growth factor-1 can prevent the metastatic and EMT processes of melanoma cells by downregulating the stem cell markers Sox2, OCT3/4, CD44 CD133 and deleting stem cell functional characteristics (97). CD44 may therefore serve an important role in melanoma metastasis.

3. Melanoma stem cells and angiogenesis

The process of angiogenesis is an important hallmark of the growth and progression of tumors, including melanoma (98). Evidence has shown that tumor vessels are derived from capillaries and veins in the host tissue, following activation by pro-angiogenic factors of pre-existing endothelial cells migrating into the tumor and developing into new vessel networks (99). The endothelial progenitor cells found in the peripheral circulation also contribute to tumor angiogenesis by differentiating and proliferating in a local tumor (100). Similarly, mesenchymal stem cells promote the growth and angiogenesis of tumors thanks to their self-renewal capacity, long-term viability and differentiation potential toward diverse cell types (101-104). For example, $CD133^+$ glioma cells secrete higher levels of VEGF than $CD133^-$ cells, which indicates that tumor stem cells promote tumor angiogenesis (105). Furthermore, MSCs, which are involved in tumor angiogenesis, can accelerate melanoma metastasis. The renal and melanoma derived-CSCs are able to differentiate into endothelial like cells when cultured in endothelial cell growth specific medium (26,106). In addition, melanoma cells with stem cell-like characteristics, particularly those locating at the margin of the tumor, such as melanoma initiating cells, express and deliver in the microenvironment several factors (including VEGF, bFGF and PDGF) associated with angiogenesis (20). Since MSCs have high degree of differentiation plasticity, they can contribute to the *de novo* formation of tumor angiogenesis via a process named vasculogenic mimicry (VM) (107). Interestingly, tumor cells with abundant VM have a high plasticity, and their ability to mimic vascular endothelial cells may be related to the stemness of tumor cells (107,108). It has been reported that increasing the expression of stem cell-like genes in melanoma can improve the plasticity of tumor cells (108,109). Furthermore, $ABCB5^+$ MSCs express specific endothelial and proangiogenic factors as well as VE-cadherin, Tie2, VEGF and its receptors, which are specific markers of VM (46). Consistently with these observations, melanoma cells expressing the CD133 and ABCG2 stem cells markers overexpress proangiogenic proteins, such as VEGF and its receptor VEGFR-2, Tie2 and angiopoietin (27). Furthermore, $CD271^+$ MSCs were demonstrated to be associated with VM, through activation of the VEGR receptor/PKC signaling pathway (64,110). Thus, MSCs in angiogenesis contribute to melanoma growth and metastasis.

4. Targeting molecular markers of melanoma stem cells

The molecular markers of MSCs are of relative specificity and can serve as targets of molecular targeted therapy. They not only contribute to the removal of MSCs but also prevent normal cells from being damaged, in order to achieve the highest benefit of tumor therapy. For example, tubacin, which is an inhibitor of histone deacetylase 6, can promote the release of $CD133^+$ exosomes in the human metastatic melanoma cells FEMX-I, decrease the content of CD133 in cells and inhibit the proliferation and clonogenesis of FEMX-I cells (111). In addition, monoclonal antibodies against different epitopes of CD133 have exhibited a dose-dependent cytotoxicity in FEMX-I cells (28,112). Furthermore, CD133 monoclonal antibody can inhibit the proliferation of FEMX-I melanoma cells and prevent the growth of melanoma through its cytotoxic effect. It was also demonstrated that CD133 downregulation by shRNA can decrease the appearance of lung and spinal cord metastases from melanoma (28). In addition, andrographolide can block the expression of notch1-dependent CD133 in melanoma cells and decrease the activation of MAPK signaling pathway, leading thus to inhibition of tumor growth, angiogenesis and metastasis (26). Similarly, the elimination of chemoresistant ABCB5-positive cells may significantly inhibit the overall growth of xenotransplanted melanomas by a selective antibody (37). ABCB5 is involved in the *in vitro* and *in vivo* survival of melanoma cells following exposure to dacarbazine and the BRAF inhibitor vemurafenib (113), and

systemic administration of anti-ABCB5 antibody inhibits melanoma tumorigenesis in nude mice (37). Furthermore, cuprous oxide nanoparticles can significantly decrease the expression of Sox10 and CD271, which accelerates the apoptosis and inhibits the tumorigenicity of CD271 overexpressing A375 and WM266-4 cells (114). Lunasin can decrease the expression level of ALDH, a marker of MSCs, and the expression level of Nanog, a stem cell-related factor. Lunasin can also promote the upregulation of microphthalmia associated transcription factor that inhibits the colony-forming ability of tumor cells and the growth of xenograft tumors, and can increase the transformation from $ALDH^{high}$ to $ALDH^{low}$ melanoma cells (115). Furthermore, magnolol can decrease the expression of CD271, CD166, JARID1B and ABCB5 through reducing the expression level of notch 2, a downstream target protein of HES-1, and of the cell cycle-related protein cyclin D1, inhibiting therefore the proliferation of melanoma cells and inducing their autophagy (116). In a clinical trial, rituximab, an anti-CD20 monoclonal antibody, was used to treat $CD20^+$ metastatic melanoma. The anti-CD20 monoclonal antibody can eliminate $CD20^+$ melanoma cells and increase the level of peripheral B cells in patients with melanoma (77). In an *in vivo* and *in vitro* study of melanoma cells, MSCs-induced angiogenesis mimicry was shown be a potential biological target for some anticancer compounds, such as the natural phytochemicals lupeol, which can prevent tumor metastasis by inhibiting angiogenesis (117). However, current research mainly uses cell and animal models, and large-scale clinical trials are required to support these conclusions.

5. Conclusion

The present review on MSCs provided some insights into the metastasis, recurrence, drug resistance and treatment of melanoma. MSCs serve a crucial role in the occurrence and progression of melanoma, especially in tumor metastasis. MSCs can promote tumor progression via MSCs-specific markers and the subsequent regulation of related signaling pathways. It is therefore crucial to fully understand the underlying mechanisms of MSCs markers in the process of tumor metastasis, which would allow the discovery of effective targets for the targeted therapy of melanoma. By exploring MSCs markers and the related signal transduction pathways, we believe that effective treatment strategies to inhibit tumor metastasis or eradicate melanoma might be discovered in the near future.

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Authors' contributions

QY drafted the initial manuscript, and edited and critically revised the manuscript. HJ and DW gave guidance on the conception and design of the review. XS and SL were involved in conceiving and designing the review. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Conmpeting interests

The authors declare that they have no competing interests.

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