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REVIEW

Novel biomarkers and therapeutic targets for optimizing the therapeutic management of melanomas

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Abstract

Cutaneous malignant melanoma is the most aggressive form of skin cancer with an extremely poor survival rate for the patients diagnosed with locally invasive and metastatic disease states. Intensive research has led in last few years to an improvement of the early detection and curative treatment of primary cutaneous melanomas that are confined to the skin by tumor surgical resection. However, locally advanced and disseminated melanomas are generally resistant to conventional treatments, including ionizing radiation, systemic chemotherapy, immunotherapy and/or adjuvant stem cell-based therapies, and result in the death of patients. The rapid progression of primary melanomas to locally invasive and/or metastatic disease states remains a major obstacle for an early effective diagnosis and a curative therapeutic intervention for melanoma patients. Importantly, recent advances in the melanoma research have led to the identification of different gene products that are often implicated in the malignant transformation of melanocytic cells into melanoma cells, including melanoma stem/progenitor cells, during melanoma initiation and progression to locally advanced and metastatic disease states. The frequent deregulated genes products encompass the oncogenic B-RafV600E and N-RasQ61R mutants, different receptor tyrosine kinases and developmental pathways such as epidermal growth factor receptor (EGFR), stem cell-like factor (SCF) receptor KIT, hedgehog, Wnt/β-catenin, Notch, stromal cell-derived factor-1 (SDF-1)/CXC chemokine receptor-4 (CXCR4) and vascular endothelial growth factor (VEGF)/VEGFR receptor. These growth factors can cooperate to activate distinct tumorigenic downstream signaling elements and epithelial-mesenchymal transition (EMT)-associated molecules, including phosphatidylinositol 3'-kinase (PI3K)/Akt/ molecular target of rapamycin (mTOR), nuclear factor-kappaB (NF-κB), macrophage inhibitory cytokine-1 (MIC-1), vimentin, snail and twist. Of therapeutic relevance, these deregulated signal transduction components constitute new potential biomarkers and therapeutic targets of great clinical interest for improving the efficacy of current diagnostic and prognostic methods and management of patients diagnosed with locally advanced, metastatic and/or relapsed melanomas.

Key words: Cutaneous melanomas; Melanoma stem/progenitor cells; Biomarkers; Screening tests; Diagnosis; Prognosis; Molecular targets; Targeted therapy

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INTRODUCTION

Cutaneous malignant melanoma represents the major cause of mortality among skin cancers and its incidence rate is increasing during last years[1-5]. Although the localized cutaneous melanomas diagnosed in the early stages are usually curable by surgical resection of malignant tumors, the rapid progression to invasive and metastatic disease states is generally associated with a poor median survival of 6 mo to 12 mo and a five year survival rate of less than 10%[1,2,6-8]. The therapeutic options for the patients with unresectable melanomas and metastases at distant organs such as lungs, liver and brain consisting to the radiation therapy and/or chemotherapy are only palliative, aiming to improve the quality of life of patients[8-10]. Especially, the standard treatment with alkylating agent, dacarbazine or its orally active analog temozolomide, alone or in combination with other cytotoxic agents, is ineffective in the most cases and culminate to the development of drug resistance, disease relapse and the death of melanoma patients[11-13].

Importantly, recent advances in melanoma research have led to the establishment of the molecular oncogenic events that may contribute to melanoma initiation and progression and treatment resistance of melanoma cells. It has been observed that the persistent activation of different oncogenic signaling cascades initiated in an autocrine or a paracrine manner by distinct growth factors and cytokines through their cognate receptors is typically involved in the sustained proliferation, survival, invasion and metastases at near lymph nodes and distant sites of melanoma cells and angiogenic process[2,14-23]. These deregulated gene products include B-Raf^{V600E}, N-Ras^{Q61R}, epidermal growth factor receptor (EGFR), hepatocyte growth factor (HGF) receptor MET, platelet-derived growth factor receptors (PDGFRs), sonic hedgehog, Wnt/β-catenin, Notch, Nodal/Cripto, hyaluronan (HA)/CD44, stem cell-factor (SCF) receptor KIT, stromal cell-derived factor-1 (SDF-1)/CXC chemokine receptor-4 (CXCR4), and vascular endothelial growth factor (VEGF)/VEGFR receptor (Figure 1)[13,14,17,19,22,24-47]. These tumorigenic pathways can cooperate for the sustained activation of downstream signaling effectors such as mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3'-kinase (PI3K)/Akt, nuclear factor-kappaB (NF-κB) and hypoxia-inducible factors (HIFs) for the acquisition of a more malignant behavior by melanoma cells during disease progression to locally advanced and metastatic states.

In addition, recent advances in skin stem/progenitor cell research have led to the identification of melanoma cells endowed with stem cell-like properties and which can provide critical functions for tumor growth, metastases at distant sites, treatment resistance and disease relapse[17,18,20,21,23,48,49]. More specifically, highly tumorigenic melanoma stem/progenitor cells have been identified *in situ* and isolated from primary and secondary melanoma tumors, circulating melanoma cells and established melanoma cell lines[20,21,50-64]. Melanoma stem/progenitor cells may express different stem cell-like markers such as CD133, nestin, aldehyde dehydrogenase (ALDH-^{high}), CD166, neural crest nerve growth factor receptor (CD271) and/or ATP-binding cassette (ABC) multidrug resistance transporters such as multidrug resistance-1 encoding P-glycoprotein (P-gp), ABCG2 and ABCB5. It has been shown that highly tumorigenic melanoma stem/progenitor cells can give arise to the total tumor cell mass *in vivo* with the phenotypic features resembling to original patient's melanomas and metastasize at distant sites[50-58,60,61,63-65]. In this matter, we review the most recent advancements on the gene products that are often altered during melanoma initiation and progression to locally invasive and metastatic disease states and which may be exploited to develop novel multiplex biomarker detection methods for optimizing diagnosis and prognosis and multitargeted therapies for a more effective management of melanoma patients.

NEW BIOMARKERS FOR OPTIMIZING DIAGNOSIS, PROGNOSIS AND INDIVIDUALIZED TREATMENT OF MELANOMA PATIENTS

The clinical diagnosis of cutaneous malignant melanomas at early stages retains a big challenge for the experimented pathologists and is generally made only after they become visible on skin[66]. Moreover, a skin biopsy and different tumor imaging tests such as X-rays, computed tomography (CT) scan, magnetic resonance imaging (MRI) and positron emission tomography (PET) tests are often performed to establish the grades and stages of melanomas and screen for metastatic melanomas[66,67].

In addition, the immunohistochemical staining of tissue specimens with different antibodies directed against different melanocytic markers such as S-100 and melanoma-associated antigen recognized by T-cells (MART-1) also designated as melanocyte antigen (Melan-A), which is expressed by melanoma cells, is useful for improving the accuracy of the pathological diagnosis and prognosis of melanoma patients[68-70]. Moreover, monoclonal antibody gp100 corresponding to clone HMB-45, which is highly specific and sensitive for melanocytic tumors but does not react with other non-melanoma malignancies such as carcinomas, lymphomas and sarcomas and normal melanocytes, may be used for the pathological diagnosis to distinguish poorly differentiated melanoma subtypes of other tumor types[69,71]. The immunohistochemical analysis of the vimentin expression in primary melanoma tissues, which is frequently overexpressed in primary melanoma patients with hematogenous metastasis, also may help to establish the melanoma patients with a high risk to develop hematogenous metastasis[72]. Although this importance advance, few biomarkers in melanoma stem/progenitor cells and their progenies have been validated in the clinics to use in combination in screening methods for an early

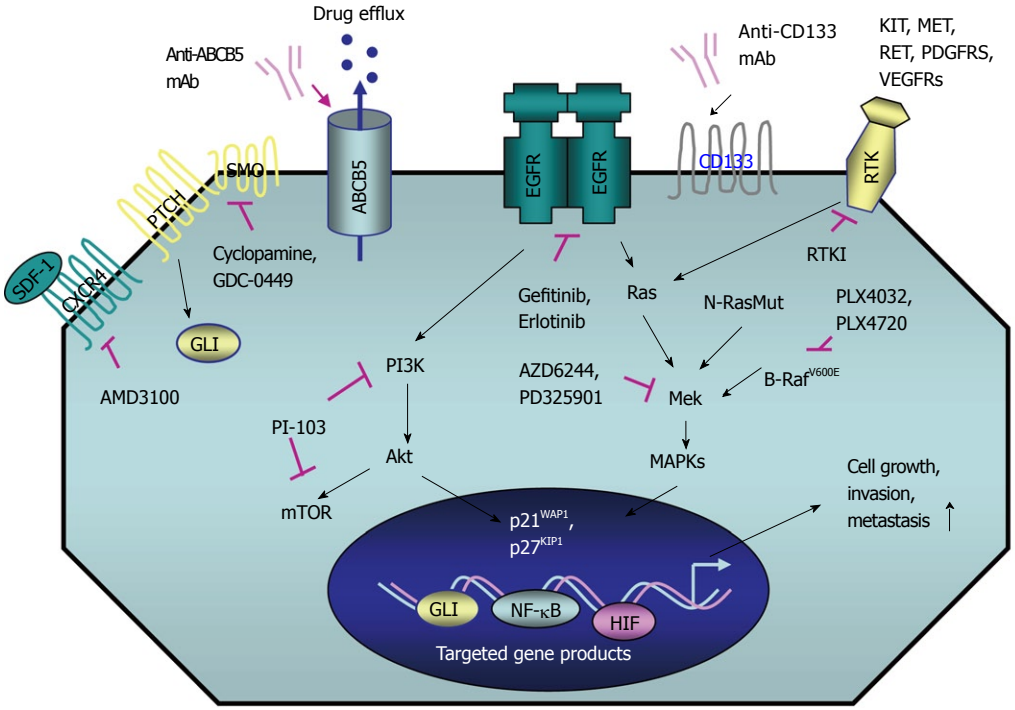

Figure 1 Novel multitargeted strategies against locally advanced, aggressive and metastatic melanomas. The scheme shows the intracellular signaling cascades induced through the activation of distinct growth factor pathways which may provide critical roles for the sustained growth, survival, migration, invasion, metastases and/or drug resistance of melanoma cells, including melanoma-initiating cells, through the up-regulation of the expression levels of different oncogenic gene products. The oncogenic gene products include c-Myc, Bcl-2, N-cadherin, snail, twist, matrix metalloproteinases (MMPs), urokinase-type plasminogen activator (uPA), cyclooxygenase-2 (COX-2) and vascular endothelial growth factor (VEGF). The potential therapeutic agents that may be used to block these tumorigenic signaling pathways, including a selective inhibitor of receptor tyrosine kinases (RTKI), EGFR (gefitinib or erlotinib), smoothened (SMO) hedgehog signaling element (cyclopamine or GDC0049) PI3K/mTOR (PI-103), oncogenic B-Raf^{E600V} mutant (PLX4032 or PLX4720) and MEK (AZD6244) as well as a monoclonal antibody (mAb) directed against stem cell-like markers, CD133 and ABCB5 multidrug transporter are also indicated. NF-κB: Nuclear factor-kappaB; HIF: Hypoxia-inducible factors; EGFR: Epidermal growth factor receptor.

and non-invasive detection of cutaneous melanomas and the establishment of the risk of the disease progression, metastases at near lymph nodes and distant sites and relapse. Consequently, the identification and validation of novel molecular biomarkers associated with the melanoma initiation and progression to locally invasive and metastatic disease states and response of melanoma patients to the clinical treatment is of great interest for improving the efficacy of current diagnostic and prognostic methods and therapeutic management of melanoma patients.

Numerous cytogenetic analyses in malignant melanoma tissues and serum samples *vs* benign melanocytic naevi and normal tissues and serum samples using microarray, immunohistochemical and polymerase chain reaction (PCR)-based techniques have led to the discovery of novel deregulated genes in melanoma cells[18,23,42,73-83]. The gene products often altered during melanoma progression constitute potential biomarkers for a more early diagnosis and accurate prognosis of melanoma patients and effective personalized medicine. The potential biomarkers that may be detected in malignant tissues and/or serum samples, either alone or in combination, to establish the risk of disease progression and as prognostic indicator of melanoma patients include different oncogenic products. Among the more promising molecular biomarkers, there are EGFR, activated pAkt phosphorylated form, microphthalmia-associated transcription factor (MITF), serum amyloid, MIC-1 also designated as growth and differentiation factor-15 (GDF-15), VEGF, interleukin-8 (IL-8) and/or twist[18,23,42,73-79,84-88].

More specifically, it has been observed that the EGFR expression was enhanced in primary and metastatic melanoma tissues from patients relative to non-malignant tissues suggesting that the detection of EGFR could be used as a prognostic indicator to predict the risk of disease progression to metastatic disease states and poor outcome of melanoma patients[86;87]. Moreover, the overexpression of MITF protein has also been detected in 62 of 104 tumor tissues obtained from metastatic melanoma patients and correlated with the chemotherapeutic response and reduced disease-specific survival of melanoma patients[73]. Importantly, the secreted MIC-1 cytokine has also been observed to be overexpressed in 66% of 53 melanoma cell lines analyzed as compared to normal melanocytes[76]. Moreover, the immunohistochemical analyses have indicated that the MIC-1 protein was expressed at low levels in primary melanoma biopsies (15 of 22) while all metastatic melanoma biopsies examined (16 of 16) exhibited strong expression of MIC-1[76]. The results from another study have also indicated that

MIC-1 was overexpressed in approximately 67% cases of advanced melanomas and secreted MIC-1 protein levels detected in serum samples of melanoma patients were 5-6 fold higher as compared with serum samples from normal individuals[42]. In this matter, it has been reported that the enhanced MIC-1 expression in melanoma cells may be induced at least in part through the constitutively active mutant B-Raf^{V600E} and activation of MAPKs, and to a lesser extent *via* the activated PI3K/Akt pathway[42,76]. The stimulation of SCF receptor KIT, which may contribute to the activation of MAPK pathway and the phosphorylation of MITF, also may result in an up-regulation of the MIC-1 expression[76]. Hence, together these results combined with the fact that the secreted MIC-1 cytokine has been observed to promote the tumorigenicity of melanoma cells *in vivo*[42;76], support the clinical interest to detect MIC-1 in melanoma tissue biopsies or serum samples for improving the diagnosis and prognosis of melanoma patients.

On the other hand, the occurrence of polymorphisms in the melanocortin-1-receptor (*MC1R*), which may lead to the *MC1R* variants encoding a non-functional MC1R protein and the acquisition of a red hair color (RHC) phenotype, fair skin, freckles and poor tanning ability of individuals, has also been associated with a high risk of developing melanoma[89]. Interestingly, the combined immunohistochemical analyses of expression levels of different cell cycle modulators (p21, p27, p53 and retinoblastoma proteins) and pro-apoptotic factors (Bax and Bak) in primary cutaneous melanoma tissues at stage Ⅱa from 31 patients performed during a 10-year follow-up period have also indicated that the down-regulation of these markers may be more appropriate than the detection of a single molecular marker for assessing the risk of melanoma progression and metastases[83].

Of particular therapeutic interest, a multicenter phase Ⅱ trial has also been undertaken in order to investigate the efficacy of a sensitivity-directed, first-line chemotherapy in patients with metastasized melanomas by performing an *in vitro* assay using an ATP-based luminescence viability test for evaluating the chemosensibility of viable melanoma cells obtained from metastatic lesions to seven single drugs and five drug combinations[90]. The results have revealed that among the 53 patients evaluable for all study end points, 22 (42%) were chemosensitive and 31 (58%) chemoresistant patients and the chemosensitive patients showed an increased overall survival of 14.6 months compared with 7.4 mo in chemoresistant patients[90]. In the same way, the results from a recent study have also indicated the possibility to establish the B-Raf^{E600V} mutation status in the tissue biopsies and circulating free DNA samples from melanoma patients to assess the patients that could be susceptible to respond to the pharmacological agents targeting oncogenic B-Raf^{E600V} mutant[91]. In addition, it has also been noted that the serum concentrations of diverse angiogenic factors such as VEGF, basic fibroblast factor (bFGF) and IL-8 were increased in melanoma patients relative to healthy individuals and associated with advanced stages and poor overall and progression-free survival of melanoma patients[18,23]. More particularly, a study carried out with 35 patients with stage Ⅳ melanoma has indicated that 15 patients who responded to chemotherapy showed a significant decrease in the serum IL-8 level while non-responders with progressive disease did not[92]. These data suggest that the detection of serum IL-8 level could serve as an indicator of the potential response of melanoma patients to the chemotherapeutic treatment.

Potential biomarkers in melanoma stem/progenitor cells

Of great clinical interest, the results from recent studies have also indicated the possibility to detect the stem cell-like markers such as ABCB5, nestin, CD133 and CD166 in primary and metastatic melanoma tissue specimens and/or circulating melanoma stem/progenitor cells in combination with current clinical biomarkers to predict the risk of the metastasis formation and overall survival of melanoma patients[51,55,59,63,93-95]. For instance, it has been observed that highly tumorigenic circulating melanoma cells isolated from the peripheral circulation of melanoma patients expressing the stem cell-like marker, ABCB5 multidrug transporter were tumorigenic and able to form the metastases in animal model *in vivo*[63]. Furthermore, it has been observed that the expression of ABCB5 protein was enhanced in primary and metastatic melanoma specimens as compared to normal skin and benign nevi[51,55,94]. Then, these data support the interest to detect the ABCB5 multidrug transporter in primary melanoma tissue specimens and circulating melanoma cells to predict the risk of progression to metastatic disease states.

The immunohistochemical analysis of nestin, which is a neuroepithelial intermediate filament expressed in proliferative neuroectodermal progenitor cells during embryonic development and adult bulge areas-resident stem cells in hair follicle, has also indicated that its expression was significantly enhanced in primary and metastatic melanoma tissue specimens as compared to benign and normal melanocytes[96-101]. Nestin was also co-expressed with SOX9 and SOX10, which may contribute to its transcriptional up-regulation, in primary and secondary melanoma specimens and associated with a poor survival of melanoma patients[98-101]. The analyses by flow cytometry and quantitative reverse transcription-PCR (qRT-PCR) of the expression level of nestin performed on 23 tissue specimens from patients with stage Ⅲ-Ⅳ melanoma has also indicated that this stem cell-like marker was expressed at a higher level in stage Ⅳ patients compared to stage Ⅲ/Ⅳ with no evidence of disease[93]. It has also been noted that the expression of nestin positively correlated with the tumor burden and tyrosinase and melan-A co-expression in malignant tissues[93]. Nestin has also been detected with tyrosinase in a proportion of circulating melanoma cells enriched from peripheral blood samples while no cells expressing nestin were detected in peripheral blood of healthy volunteers[93].

Additionally, it has also been reported that the percentage of circulating melanoma cells expressing stem cell-like markers, nestin and CD133, detected in 32 melanoma patients correlated with tumor burden and number of metastatic sites, and was associated with a shorter overall survival of patients[59]. The immunohistochemical analyses of co-expression of different stem cell-like markers, including nestin, CD133, ABCB5 and CD166 have also indicated that these biomarkers were significantly enhanced in primary and metastatic melanoma specimens as compared to melanocytic nevi[102,103]. On the other hand, a higher proportion of melanoma cells coexpressing stem cell-like markers, CD271 and SOX10, has also been detected within melanoma biopsies of primary tumors, melanoma metastases and melanoma cell lines and associated with higher metastatic potential and poor tumor-specific survival of melanoma patients[64].

Collectively, the recent advancements on the identification of distinct potential biomarkers in melanoma stem/progenitor cells and their differentiated progenies offer now the possibility to assess their expression levels in primary and metastatic melanoma tissue specimens, serum samples and/or circulating melanoma cells detected in peripheral circulation from patients in the clinics. The simultaneous analyses of the expression of these novel molecular biomarkers could be exploited to develop more effective and non-invasive screening tests for improving the current diagnostic and prognostic methods. Moreover, these novel molecular biomarkers could be used to predict the potential response of melanoma patients to the inhibitory agents targeting these deregulated signaling elements, and thereby lead to an optimization of the choice of cytotoxic drugs for their therapeutic treatment in the clinics. In this matter, we review data from recent *in vitro* and *in vivo* studies and clinical trials carried out to validate new potential therapeutic targets in melanoma stem/progenitor cells and their progenies for improving current treatments of patients diagnosed with aggressive melanomas.

NEW THERAPEUTIC STRATEGIES AGAINST AGGRESSIVE AND METASTATIC MELANOMAS

Molecular targeting strategies

Recent investigations in melanoma research have led to the identification of several molecular pathways and specific gene products that are often deregulated during melanoma initiation and progression to locally advanced and metastatic disease states. The oncogenic products constitute new potential therapeutic targets to eradicate the total melanoma cell mass, including melanoma stem/progenitor cells, and prevent disease progression and relapse. These deregulated gene products include B-Raf^{V600E}, N-Ras^{G61K}, different receptor tyrosine kinases (RTKs) such as EGFR, KIT, MET, PDGFRs and VEGFRs as well as sonic hedgehog, Wnt/β-catenin, Notch, Nodal/Cripto, HA/CD44 and SDF-1/CXCR4 and their downstream signaling effectors such as PI3K/Akt, NF-κB and MIC-1 as well as ABC multidrug resistance transporters (Figure 1; Table 1)[13,17,22,24-46]. The blockade of these tumorigenic pathways and targeting of drug resistance-associated molecules by using specific inhibitory agents has been shown to suppress the growth, invasion and/or metastases of melanoma cells and angiogenesis process *in vitro* and *in vivo*[17,24-46,104]. For instance, a combination of EGFR tyrosine kinase inhibitor, erlotinib plus adenoviral vector-mediated IL-24 expression was more effective as individual agents at inhibiting growth and inducing apoptosis of different melanoma cell lines *in vitro*[105]. In the same way, the combined treatment with erlotinib and a monoclonal antibody (mAb) termed bevacizumab that binds to and inhibits VEGF, also induced supra-additive inhibitory effect on the tumor growth of melanoma cell-derived xenografts and reduced the metastatic spread of melanoma cells to lymph nodes and lungs in mice as compared to single agents[106]. The antitumoral effects of the combined drugs was mediated in part through the inhibition of proliferation and increase of apoptosis of melanoma cells as well as a reduction in tumor angiogenesis[106]. Moreover, it has been reported that the activation of Ras/MAPK and PI3K/Akt pathways may contribute to the up-regulation of GLI transcriptional effector of hedgehog cascade in melanoma cells and the inhibition of smoothened (SMO) co-receptor for sonic hedgehog ligand using cyclopamine reduced the growth of melanoma cell-derived xenografts and metastases in mice and prevented disease recurrence (Figure 1)[104].

Importantly, the targeting of the stem cell-like marker CD133 using mAbs has also been reported to induce the cytotoxic effects in FEMX-I melanoma cells *in vitro* and reduce their metastatic spread in mice *in vivo*[57]. Moreover, the inhibition of ABCB5 multidrug transporter using a mAb also inhibited the tumor growth of CD133$^+$/ABCB5$^+$ melanoma stem cell-derived xenografts *in vivo*[55]. A combination of a CXCR4 inhibitor AMD3100 plus current chemotherapeutic drug, darcarbazine was also more effective at reducing the tumor growth and metastases of chemoresistant CD133$^+$/CXCR4$^+$ melanoma cells *in vivo* as compared to single drugs[65]. Additional studies, however, are necessary to further establish the molecular mechanisms at the basis of the cytotoxic effects of these therapeutic agents, alone or in combination therapies with current chemotherapeutic drug, dacarbazine on different melanoma cell models.

In addition, several clinical trials have also been carried out or are undergoing to investigate the anticarcinogenic efficacy of new chemopreventive and anticarcinogenic agents and diverse immunosuppressive therapeutic strategies such as the use of dentritic cells, high-doses of interferon-α (IFN-α) and/or IL-2 and anti- cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) antibody, alone or in combination with current therapies for treating locally advanced, metastatic and recurrent melano-

Table 1 Potential therapeutic targets in melanoma stem/progenitor cells and their progenies

Targeted deregulated element	Name of inhibitory agent
mAb against stem cell-like surface marker	
CD133	Anti-CD133 mAb
ABCB5	Anti-ABCB5 mAb
Growth factor signaling inhibitor	
EGFR (erbB1) antibody	mAb-C225, cetuximab (IMC-C225), IMC-1121B
EGFR-TKI	Gefitinib, erlotinib, AG1478, PD153035
Anti-EGF antibody	ABX-EGF
Pan-erbB1/erbB2/erbB3/erbB4-TKI	Cl1033
MET	SUI1274
Hedgehog	Anti-SHH antibody, SMO inhibitor (cyclopamine, GDC-0449, BMS-833923, NVP-LDE225, IPI-926 IPI-269609)
Wnt/β-catenin	Anti-Wnt antibody, WIF-1
Notch	γ-secretase inhibitor (DAPT, MK-0752,GSI-18)
Nodal/Cripto	LEFTY, Anti-Cripto mAb
KIT	Imatinib mesylate, dasatinib
HA/CD44	Anti-CD44 mAb, soluble CD44 protein
VEGF	Anti-VEGF antibody (bevacizumab)
VEGFR2	Anti-VEGFR-2 mAb (DC101)
VEGFR2/EGFR/RET	Vandetanib (ZD6474)
VEGFRs, PDGFRs, KIT	Sunitinib
B-Raf, C-Raf, KIT, PDGFRs, VEGFR2 and 3	Sorafenib
ECM component/integrin	Anti-integrin antibody
CXCR4	AMD3100
Intracellular signaling inhibitor	
B-RafE600V	PLX 4032, PLX4720
MEK1/2	AZD6244 (ARRY-142886), PD0325901
PI3K	LY294002
mTOR	Rapamycin, CCl-779,
PI3K/mTOR	PI-103
NF-κB	IkBα inhibitor, sulfasalazine, bortezomib (PS-341) salinosporamides A (NPI-0052), parthenolide
COX-2	NS-396, etodolax, celecoxib, rofecoxib
Immunomodulatory agent	
Immune and/or vascular systems	Imiquimod, INF-α, IL-2, IL-21, anti-CTLA-4, mAb (ipilimumab "MDX 010"and tremelimumab "CP-675, 206")

CTLA-4: Cytotoxic T lymphocyte-associated antigen 4; DAPT: N-(N-3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; ECM: Extracellular matrix; EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; IκBα: Inhibitor of nuclear factor-κBα; IL: Interleukin; INF: Interferon; mAb: Monoclonal antibody; NF-κB: Nuclear factor-kappaB; PDGFRs: Platelet-derived growth factor receptors; MET: Hepatocyte growth factor receptor; PI3K: Phosphatidylinositol 3'-kinase; SMO: Smoothened; TKI: Tyrosine kinase inhibitor; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; WIF-1: Wingless inhibitory factor-1; Wnt: Wingless ligand.

mas[33,66,107-119]. The cytotoxic drugs include the specific inhibitors of B-Raf^{E600V}, N-Ras^{G61K}, KIT, EGFR and hedgehog signaling elements. Importantly, the results from phase I clinical studies with a orally active inhibitor of oncogenic B-Raf^{V600E} product, PLX4032 carried out with 32 patients with metastatic melanomas harboring the B-Raf^{V600E} mutation have revealed that this treatment led to a substantial tumor regression including 24 patients that showed a partial response and 2 had a complete response[120]. The trials are now undergoing to determine the long-term effect of PLX4032, alone or in combination with other agents such as MEK inhibitor, on the survival of melanoma patients[121,122]. In regard with this, the clinical responses have also been observed in a phase II study with orally active and highly selective inhibitor of MEK1/2, AZD6244 (ARRY-142886) or temozolomide performed with 200 patients with advanced melanoma harboring B-Raf^{E600V} mutation[123,124]. On the other hand, it has also been reported that the melanoma patients harboring the activating mutations in the KIT receptor exhibited a partial or complete response to imatinib mesylate[33]. It has however been noted that dasatinib was more effective than imatinib at reducing the viability of melanoma cells in two melanoma patients harboring the KIT^{L576P} mutation which is the most frequent KIT mutation occurring in approximately 30%-40% cases of melanoma[44]. Furthermore, the data from a multi-institutional phase II trial with an oral multikinase inhibitor termed sorafenib, which targets different tyrosine protein kinases, including wild-type and mutant B-Raf and C-Raf kinases, PDGFRs, KIT, VEGFR2 and VEGFR3, performed with 36 patients with advanced melanomas have indicated that 1 patient showed a partial response for 175 d and 3 patients had stable disease with a mean duration of 37 wk[125].

Immunotherapy-based strategies

Among other promising experimental strategies, the results from the clinical trials with a experimental treatment consisting to a topical application of a cream containing 5% an immunomodulatory agent, imiquimod (Aldara) after surgical excision of tumors have revealed that this treatment reduced some melanocytic nevi and melanoma-in-situ (lentigo maligna)[9,10,107-109,126,127]. Moreover, the data from a Phase Ⅰ/Ⅱ study of a combination of topical imiquimod and intralesional IL-2 have also revealed that its treatment induced a significant clinical response in patients with multiple accessible melanoma metastases by increasing the activated lymphocytes and the production of IFN-γ by peripheral blood mononuclear cells as well as by restoring the Th1/Th2 balance[110,111]. In addition, a therapeutic treatment consisting of an adjuvant immunotherapy with high doses of immunosuppressive agents, IL-2 and/or IFN-α, alone or in combination with chemotherapy or adoptive cell therapy, has also been observed to result in a complete and long-lasting remission in a small subset of melanoma patients[1,9,10,66,113,116,117,128-130]. In particular, it has been reported that the melanoma cell density in metastases and angiogenesis was significant reduced after a treatment with IFN-α[131]. Moreover, the results of phase Ⅱ trials with 28 patients with stage Ⅳ

melanoma without brain metastases have revealed that a combination of dacarbazine plus pegylated IFN-α2a was well tolerated and associated with a response rate of 24% in 25 patients evaluable for response, including 2 long-lasting complete responses[132]. Interestingly, the results of a phase II trial with an oncolytic herpes simplex virus type 1 encoding granulocyte macrophage-colony stimulating factor (GM-CSF), designated as Oncovex (GM-CSF), have also indicated a 28% objective response rate occurred in patients with melanomas which was accompanied by a tumor regression of both injected and non-injected lesions[126,127]. These data suggest that the treatment with Oncovex (GM-CSF) can induce a direct oncolytic effect in injected tumors as well as a secondary immune-mediated anti-tumor effect on non-injected tumors[126,127].

CONCLUSION

Significant advancements made in last few years have provided important information on the molecular signaling pathways and gene products that are frequently deregulated in melanoma stem/progenitor cells and their progenies during melanoma formation and progression to locally advanced and metastatic disease states. Consequently, the combination of different molecular biomarkers or cytotoxic agents targeting distinct gene products altered during melanoma development may constitute more promising therapeutic strategies as the use a single biomarker or monotherapy for improving the accurate of current diagnostic and prognostic methods and efficacy of the treatment of melanoma patients.

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